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NEWS EXPRESS	MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
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FILE 'HOME' ENTERED AT 14:24:02 ON 04 JUN 2004

=> file .biotech caplus
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.42	0.42

FILES 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 14:24:57 ON 04 JUN 2004

7 FILES IN THE FILE LIST

=> s hofman s?/au
L1 88 HOFMAN S?/AU

=> s double stranded DNA or dsDNA
L2 205543 DOUBLE STRANDED DNA OR DSDNA

=> s l2 and (forward primer and reverse primer)
L3 13 L2 AND (FORWARD PRIMER AND REVERSE PRIMER)

=> s l3 and captur
=> s l3 and captur?
L4 2 L3 AND CAPTUR?

=> d ibib abs l4 1-2

L4 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-04910 BIOTECHDS
TITLE: Analysis of polynucleotides in a sample using generic **capture** sequences comprises amplifying target polynucleotide, and utilizing the product to indirectly assay the sample for the polynucleotide;
DNA amplification useful for pharmacogenetics, forensics, anthropology, paternity testing, functional genomics, genetic analysis, SNP, immunoglobulin mutation, pathogen detection, drug resistance, DNA sequencing anddiagnosis
AUTHOR: LAI J H; PHILLIPS V E; WATSON A R
PATENT ASSIGNEE: QUANTUM DOT CORP
PATENT INFO: WO 2001083823 8 Nov 2001
APPLICATION INFO: WO 2000-US13979 28 Apr 2000
PRIORITY INFO: US 2000-200635 28 Apr 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-114152 [15]
AN 2002-04910 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Assaying, (M1) for an amplification product (AMP) from a first target polynucleotide, (TP), comprising providing a sample that is suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a **capture** sequence not present in the TP at the same position, is new.
DETAILED DESCRIPTION - A target polynucleotide, (TP), is amplified, where first primer has a tag sequence, the complement of which is formed on the opposite strand during amplification and is referred to as **capture** sequence (CS), and the opposite strand is referred to as an amplification product (AMP) has a label; probe conjugated to substrate specific to CS, is contacted with AMP to form AMP detection complex. Assaying (M1) for an AMP from a first TP, comprises providing a sample

that is suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a **capture** sequence not present in the TP at the same position, where the AMP is formed by primer extension from a template, where the template comprises a complement to the TP and a target noncomplementary region, where the **capture** sequence is a complement to the target noncomplementary region, providing a substrate that is conjugated to a first **capture** probe, contacting the sample with the **capture** probe under a first set of hybridization conditions, where the **capture** probe can bind to the **capture** sequence under the first set of hybridization conditions and determining if the first label is associated with the substrate. INDEPENDENT CLAIMS are also included for the following: (1) forming (M2) an AMP detection complex for assaying a sample for a first TP; (2) an amplification product detection complex comprising a **capture** probe polynucleotide hybridized to **capture** sequence of labeled amplification product from a TP, where the **capture** probe polynucleotide is conjugated to a substrate, where the **capture** sequence is not present in a region of the TP which is amplified and is introduced into the amplification product by copying a template polynucleotide, the template polynucleotide comprising a target noncomplementary region and a region complementary to the TP, where the target noncomplementary region was introduced into the template polynucleotide by extension of a primer hybridized to the target polynucleotide, the primer comprising the target noncomplementary region and where the **capture** sequence is complementary to the target noncomplementary region; and (3) a kit for assaying for an AMP from a TP comprises a substrate attached to a **capture** probe, a first primer comprising a 3' end a first target complementary region located at the 3' end of the first primer, and a first target noncomplementary region that is not complementary to the first TP at a position 3' of a sequence to which the first target complementary region can hybridize, a second primer, label, housing for retaining the substrate, first primer, second primer, and a label and instruction provided with the housing that describe how to use the components of the kit to assay a sample by forming an AMP from the TP using the first and second primers that comprises the label and a **capture** sequence that is complementary to the target noncomplementary in the first primer, where the **capture** sequence can bind to the **capture** probe.

BIOTECHNOLOGY - Preferred Method: In (M1) the **capture** probe is a polynucleotide, substrate is from micorsphere, chip, slide, multiwell plate, a membrane, an optical fiber, and an optionally porous gel matrix, more preferably slide. The substrate is preferably conjugated to several different **capture** probe polynucleotides having corresponding different sequences, where each of the different **capture** probes can selectively bind to a corresponding different **capture** sequence on a corresponding different AMP. The substrate is preferably a first microsphere comprising a first spectral code comprising a first semiconductor nanocrystal and first fluorescence characteristics, where the first semiconductor nanocrystal comprises: (a) a core selected from ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AIAs, AIP, AIS, Ge, Si, Pb, PObSe, PbSe, their alloy or their mixture, more preferably the core is a CdSe; (b) a shell, preferably ZnS. The AMP is produced by a process comprising incorporation of a nucleotide comprising the first label into AMP using a polymerase or extension of a primer using a polymerase to form AMP, where primer comprises the first label, where the first label comprises an agent selected from chromophore; lumiphore; fluorophore preferably semiconductor nanocrystal, fluorescent dye, lanthanide chelate, a green fluorescent protein, more preferably is a lanthanide chelate selected from europium chelate, terbium chelate and a samarium chelate; chromogen; hapten; antigen; radioactive isotope; magnetic particle; metal nanoparticle; enzyme preferably alkaline phosphatase, horseradish peroxidase, beta-galactosidase, glucose oxidase, bacterial luciferase, an

insect luciferase and sea pansy luciferase; antibody or binding portion or their equivalent; aptamer; and one member of a binding pair; or an agent selected from avidin, streptavidin, digoxigenin and biotin. The method preferably comprises determining if the first label, preferably a fluorophore, is associated with the substrate comprises applying a light source to the substrate that can excite the fluorophore, and determining if a fluorescence emission from the fluorophore occurs from the substrate. The sample is preferably assayed for the presence of AMP or to determine its amount, where AMP is produced at a detectably higher level from at least one allele of a locus having at least two alleles. When the substrate is a first microsphere, (M1) further comprises assaying the sample for containing a second, third and fourth AMP from a second, third and fourth TP, where second AMP from second TP and is further contacted under a second set of hybridization conditions with a second **capture** probe conjugated to a second microsphere, where the second **capture** probe is a polynucleotide, the second microsphere can be the first microsphere or a different second microsphere, the second microsphere is a different second microsphere comprising a second spectral code comprising second fluorescent characteristics, the second spectral code distinguishable from the first spectral code, the second **capture** probe can hybridize to the second AMP under the second set of hybridization conditions, the second AMP comprises a second label, which can be the first label when the second microsphere is a different second microsphere or can be a different second label, and determining if the second label is associated with the second microsphere, where the first and the second AMPs are produced from a single locus, or differ by a single nucleotide. The substrate is further conjugated to a second/third/fourth **capture** probe, where the second/third/fourth **capture** probe can preferentially bind to a second/third/fourth sequence on a second/third/fourth AMP, second/third/fourth comprising a second/third/fourth second/third/fourth label that can be the same as or different than the first label and/or the second label where the binding the second/third/fourth AMP to the second/third/fourth **capture** probe can be independently determined. In (M2), after altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, the sample is further contacted with the first and second primers under conditions in which the first and second primers can hybridize to the second and first primer extension products, respectively, and be extended to form several first and second primer extension products, where altering the sample conditions to allow dissociation of the first and second primer extension product from the first target polynucleotide and first primer extension product, respectively comprises heating the sample. (M2) further comprising removing single-stranded polynucleotides from the sample prior to altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, where removing single-stranded polynucleotides are removed preferably by adding a thermolabile single-stranded nuclease to the sample under conditions and for a time sufficient to digest single-stranded polynucleotides in the sample, and then heating the sample to inactivate the single-stranded nuclease. In (M2), the first TP is at least one allele of a locus comprising a second allele, and where the first primer preferentially hybridizes to and is extended from the at least one allele as compared to second allele, where first and second primers has at least one mismatch with the second allele at one of the five 3' nucleotide of the first primer, and at least one allele and the second allele differ by a single nucleotide polymorphism. The method further comprises contacting the sample with a flanking primer has a lower melting point for hybridization to the first target polynucleotide than the first primer that is complementary to the first target polynucleotide and can be extended to form a flanking primer extension product, where the flanking primer is complementary to the first target polynucleotide at a flanking position that is 5' to a position at which the first target complementary region

is complementary to the first target polynucleotide. (M2) further comprises forming a second AMP detection complex for assaying the sample for second TP. Utilizing a third and a fourth primer. Preferred Kit: The label is provided conjugated to a nucleotide which can be incorporated into the AMP, The label is provided conjugated to the second primer. the substrate is attached to a plurality of different **capture** probes, where each of said different **capture** probes is attached at an identifiable location on the substrate, where each of said different **capture** probes can preferentially hybridize to a corresponding different AMP, each of the corresponding different AMPs comprising a label that can be the same or different as the label on the other corresponding different AMPs and where the instructions further describe how to use the components to the kit to assay the sample for each of the corresponding different AMPs. M2 comprises: (a) providing a first and second primer; where first primer comprising a 3' end a first complementary region that is complementary to the first polynucleotide, first complementary region located at the 3'- end of the first primer, and a first target non complementary region that is not complementary to the first TP at a position at 3' of a sequence to which the first target complementary region can hybridize, the first primer comprising a 3'-end and a first label; (b) providing the sample the sample suspected of containing the first TP; (c) contacting the sample with the first primer under conditions in which the first target complementary region can hybridize to the first TP and the first primer can be extended to form a first primer extension product; (d) altering the sample condition to allow disassociation of the first primer extension product from the first target polynucleotide, where the 3'-end of the first primer is complementary to the first primer extension product at a position in the first extension product that is 3' to the first target complementary region; (e) contacting the sample with the first primer under conditions in which the first can hybridize to the first primer extension product and be extended to form a first extension product comprising a first **capture** sequence that is the complement of the first target non complementary region and does not exist elsewhere in the first primer extension product, where the first primer extension product is the AMP; (f) altering the sample condition to allow dissociation of the first primer extension product from the first extension product; and (g) contacting the sample with a first **capture** probe conjugated to a first substrate where the contacting takes place under conditions in which the first **capture** probe can bind to the first **capture** sequence of the second primer extension product to form an AMP detection complex.

USE - (M2) is useful for forming an AMP detection complex for assaying a sample for first TP. The method further comprises determining if the first label is associated with the first substrate, where AMP is produced at a detectably higher level from at least one allele of a locus having at least two alleles and the first substrate preferably a first microsphere comprising a first spectral code is identified by decoding the spectral code which is preferably performed prior to, simultaneously or subsequent to determining if the first label from the second primer is associated with a substrate, first TP, preferably single-stranded or **double-stranded DNA** or RNA and a polymerase, preferably DNA polymerase having reverse transcriptase activity is used to form the first primer extension product (claimed). (M1) is useful for particular polynucleotide sequences, whether based on SNPs, conserved sequences, or other features or useful in a wide variety of different applications. The method is useful for pharmacogenetic testing, such methods ca be used in a forensic setting to identify the species or individual which was the source of a forensic specimen. Polynucleotide analysis methods can also be used in an anthropological setting. Paternity testing is another area, as is testing for compatibility, between prospective tissue or blood donors and patients in need, and in screening for heredity disorders. (M1) is also useful for studying gene expression in response to a stimulus. Other applications include human

population genetics, analyses of human evolutionary history, and characterization of human haplotype diversity. The method is useful to detect immunoglobulin class switching and hypervariable mutation of immunoglobulins, to detect polynucleotide sequences from contaminants or pathogens including bacteria, yeast, viruses, for HIV subtyping to determine the particular strains or relative amounts of particular strains infecting an individual, and can be repeatedly to monitor changes in the individuals predominant HIV strains, such as the development of drug resistance or T cell tropism; and to detect single nucleotide polymorphisms, which may be associated with particular alleles or subsets of allele. Over 1.4 million different single nucleotide polymorphisms (SNPs) in the human population have been identified. The method is also useful for mini-sequencing and for detection of mutations. Any type of mutation can be detected, including without limitation SNPs, insertions, deletions, transitions, transversions, inversions, frame shifts, triplet repeat expansions, and chromosome rearrangements. The method is useful to detect nucleotide sequences associated with increased risk of diseases or disorders, including cystic fibrosis, Tay-Sachs, sickle-cell anemia, etc. The method is useful for any assay in which a sample can be interrogated regarding an amplification product from a target polynucleotide. Typical assays involve determine the presence of the amplification product in the sample or its relative amount, or the assays may quantitative or semi-quantitative. Results from such assays can be used to determine the presence or amount of the target polynucleotide present in the sample. The above methods are particularly useful in multiplex settings where several TP are to be assayed.

EXAMPLE - 80 mul of 10 micron polysciences beads were spun down per conjugation reaction. The supernatant was removed and the beads were resuspended in 100 mul pf imidazole buffer. The beads were spun down again and the supernatant removed. The beads were again washed in imidazole buffer. After the second wash, the beads were resuspended in 20 mul imidazole buffer. 2.0 mul of 100 muM oligonucleotide and 100 mul of 200 mM 1-(3-(dimethyl-amino)-propyl)-ethylcarbodiimide hydrochloride (EDC) made fresh imidazole buffer pH 7.0 were added to the beads. The beads were then incubated for 4 hours at room temperature with shaking. The beads were then spun down, the supernatant removed, and the beads were resuspended. This step was then repeated. The beads were then again spun down, the supernatant was removed and the beads were resuspended in 100 mul of water, this step was also repeated. The beads were then spun down, the supernatant removed and the beads were resuspended in 45 mul of PBS pH 7.4. An amplification reaction was carried out using IX Amplitaq Stoffel buffer (10 mM Tris-HCl, 10 mM KCl, pH 8.3, 200 muM each dNTP), 25 mM Magnesium chloride, 2.5 U Amplitaq Stoffel enzyme, 200 mM **reverse primer** (5'-biotin-TTCAGTGCCAACCGCCTCAC-3'), 100 nM **forward primer** (5'-GCAATAGGTTTTGAGGGGCATggttgtggaa gaggac-3'), 100 nm **forward primer** (5'-TTCTGGGCCACTGACTGATTTggttgtggaagagaac-3') (where target noncomplementary regions are in capital letters and target-specific region of the primer are in lowercase letters), 1 ng/mul genomic DNA. The heat denatured amplicon was added to 2.5 mul of conjugated beads in 85 mul of hybridization buffer. The beads was spun down, by supernatant removed and then resuspended in 100 mul 1.5XSSPE (225 mM NaCl), 0.1% sodium dodecyl sulfate. The bead were again spun down and the supernatant removed to wash away excess amplicon. The beads were then resuspended in 50 mul of PBS/BSA. 0.5 mul of 0.2 mg/ml streptavidin-Cy-Chrome dye was added and incubated with the beads for 1 hour at room temperature. The beads were then spun down and resuspended in 400 mul PBS pH 7.4. The beads were analyzed on flow cytometer in PBS pH 7.4. The unique **capture** sequence-tagged amplicon showed sequence-specific hybridization to its complement attached to beads. Both alleles present in the heterozygous sample could be independently amplified and identified, and heterozygous samples could be distinguished from homozygous samples. (85 pages)

L4 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:162310 CAPLUS
DOCUMENT NUMBER: 140:176229
TITLE: Diagnostic polymerase chain reaction process utilizing simultaneous **capture** and detection of amplicons
INVENTOR(S): Hofmann, Scott Daniel
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 4 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004038194	A1	20040226	US 2001-974648	20011009

PRIORITY APPLN. INFO.: US 2000-238792P P 20001006

AB A method simultaneously detects and **captures double-stranded DNA** (**dsDNA**) sequence. The method includes adding a **forward primer** and a **reverse primer** for the **dsDNA** sequence in a sample; either the **forward primer** or the **reverse primer** have a **capture** agent, and the other has a detection agent. Replication of the **dsDNA** by PCR results in a segment of **dsDNA** with one specific end designed to be **captured** onto a medium and the opposite end designed for detection. The method is intrinsic and simultaneous and lends itself to ease of purification after **capture** and prior to detection and subsequent quantification. The method also can be applied to **dsDNA** that has been reverse -transcribed from single-stranded RNA. This procedure is exemplified by detecting an HIV viral load.

=> d his

(FILE 'HOME' ENTERED AT 14:24:02 ON 04 JUN 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 14:24:57 ON 04 JUN 2004
L1 88 S HOFMAN S?/AU
L2 205543 S DOUBLE STRANDED DNA OR DSDNA
L3 13 S L2 AND (FORWARD PRIMER AND REVERSE PRIMER)
L4 2 S L3 AND CAPTUR?

=> dup rem l3
PROCESSING COMPLETED FOR L3
L5 13 DUP REM L3 (0 DUPLICATES REMOVED)

=> d ibib abs l5 1-13

L5 ANSWER 1 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:162310 CAPLUS
DOCUMENT NUMBER: 140:176229
TITLE: Diagnostic polymerase chain reaction process utilizing simultaneous capture and detection of amplicons
INVENTOR(S): Hofmann, Scott Daniel
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 4 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

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PRIORITY APPLN. INFO.: US 2000-238792P P 20001006

AB A method simultaneously detects and captures **double-stranded DNA** (**dsDNA**) sequence. The method includes adding a **forward primer** and a **reverse primer** for the **dsDNA** sequence in a sample; either the **forward primer** or the **reverse primer** have a capture agent, and the other has a detection agent. Replication of the **dsDNA** by PCR results in a segment of **dsDNA** with one specific end designed to be captured onto a medium and the opposite end designed for detection. The method is intrinsic and simultaneous and lends itself to ease of purification after capture and prior to detection and subsequent quantification. The method also can be applied to **dsDNA** that has been reverse -transcribed from single-stranded RNA. This procedure is exemplified by detecting an HIV viral load.

L5 ANSWER 2 OF 13 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-17884 BIOTECHDS
TITLE: Determining allelic DNA sequences and haplotypes in a DNA sample, comprises using primers with a single difference corresponding to a polymorphic site combined with quantitative PCR using a fluorescent readout; with use of polymerase chain reaction and DNA primer for use in polymorphism
AUTHOR: WANG Y; XI L; FINNEY M; CHEN F
PATENT ASSIGNEE: MJ BIOWORKS INC
PATENT INFO: WO 2003046206 5 Jun 2003
APPLICATION INFO: WO 2002-US38278 27 Nov 2002
PRIORITY INFO: US 2001-334046 28 Nov 2001; US 2001-334046 28 Nov 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-482525 [45]
AN 2003-17884 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Determining the presence or absence of a first allele of a DNA sequence in a DNA sample, or the presence or absence in a DNA sample of at least two allelic DNA sequence haplotypes or alternative alleles of a DNA sequence, comprising: (a) using two PCR primers having only a single difference between them; and (b) monitoring the amount of amplicon produced in each reaction by measuring fluorescent emission from a quantitation reagent, is new.

DETAILED DESCRIPTION - Determining the presence or absence of a first allele of a DNA sequence element in a DNA sample, or the presence or absence in a DNA sample of at least two allelic DNA sequence haplotypes or alternative alleles of a DNA sequence element, where the element/haplotype/allele (D) is at least seven bases in length and differs in sequence in at least one position from the second element/haplotype/allele, comprising: (a) providing a first PCR, comprising: (i) the DNA sample; (ii) a first **forward primer** (P1) comprising a 3' end having at least seven bases that are exactly complementary to the first (D); and (iii) a **reverse primer** (P2) that participates with (P1) in PCR (b) providing a second PCR comprising: (i) the DNA sample; (ii) a second **forward primer** (P3) comprising a 3' end having at least seven bases that are exactly complementary to the second (D); and (iii) a **reverse primer** (P4) that participates with (P3) in PCR; (c) incubating the first and second PCR under cycle conditions in which the primers are extended by a polymerase; (d) monitoring the amount of amplicon produced in each reaction by measuring fluorescent emission from a quantitation reagent sensitive to the amount of double-stranded polynucleotide present

in each reaction where such monitoring occurs automatically no less often than every fifth PCR temperature cycle; and (e) comparing the fluorescence in each reaction, thus determining the presence or absence of the first allele, or whether the first or second haplotyope or allele is present in the sample, is new.

BIOTECHNOLOGY - Preferred Method: The polymerase is DELTATaq or Sso7d-DELTATaq. The step of comparing the fluorescence in each reaction involves determining the total amount of fluorescence in each reaction, or determining Ct. The allelic haplotype elements are at least 5000 bp apart.

USE - The method is useful for determining the presence or absence of a first allele of a DNA sequence element in a DNA sample, distinguishing the presence or absence in a DNA sample of at least two allelic DNA sequence haplotypes, and for distinguishing the presence or absence in a DNA sample of at least two alternative alleles of a DNA sequence element (claimed). The method is useful for scoring the presence or absence of particular polymorphisms and haplotypes in a DNA sample.

ADVANTAGE - The method provides for a specific assay for a particular polymorphism, that can be set up for the cost of synthesizing 3 or 4 primer oligonucleotides, run with little more difficulty and for little more marginal cost than two small-scale PCR reactions, using only common laboratory equipment. The assay combines allele-specific PCR with some of the technology used for quantitative PCR, along with surprising observation that simple rules are sufficient to design reliable assays with little or no experiments.

EXAMPLE - A 475 bp portion of the human cytochrome P450 gene CYP2D6 (GenBank Accession M33388, nucleotides 3265-3729) was amplified using primers A1 (forward) (AGGCGCTTCTCCGTG) and A5 (reverse) (ATGTCCTTTCCCAAACCCAT), and cloned into a TA cloning vector. Three PCR primers identical in sequence to primer A1 except for a single difference at their 3' terminal bases, primers A2 (AGGCGCTTCTCCGTC), A3 (AGGCGCTTCTCCGTA) and A4 (AGGCGCTTCTCCGTT), were used to re-amplify the CYP2D6 fragment, along with **reverse primer** A5, to generate the point mutations A, C and T at nucleotide 3280 (G was present in the most common allele in most human populations). The three amplicons were cloned as above. In order to understand the effect of amplicon size, an additional **reverse primer** A6 (CTCCAGCGACTTCTTGC), was designed to generate a much smaller amplicon of 57bp. Differential amplified polymorphism (DAP) was performed using a DNA Engine Opticon Continuous Fluorescence Detection Thermal Cycler. All reactions contained the **double-stranded DNA**-dependent fluorescent dye SYBR Green I. Increase in fluorescence was used to trace the increase in DNA amount in each cycle. Opticon Monitor software was used to determine the threshold cycle for each reaction. Reactions were performed in 32 sets of duplicates, with each set containing 300 nM of one or primers A1-A4 and 300 nM of one of primers A5 and A6. Each reaction also contained 105 copies of one of the four the template DNA clones. All perfect match conditions gave much lower Ct than all mismatch conditions. Long (475bp) and short (57bp) amplicons gave very similar results. For each template and amplicon length, the Ct value for the perfect match condition was subtracted from all Ct values, and Ct values were arranged in descending order. Minimum DELTACt values were greater than 10. It has been reported that derivatives of Taq polymerase with 5'-3' exonulcease domain removed (generally termed delta-Taq) were less likely to extend a 3' mismatch. The experiment was repeated, data from both replicates were reproducible. The minimum DELTACt for any mismatch, condition relative to the perfect match on the same template was 14.9. Many of the DELTACt values would have been higher, but the experiment was terminated at 40 cycles. The experiments showed that StH was effective at distinguishing correctly base-paired primers from incorrectly base-paired primers. (36 pages)

L5 ANSWER 3 OF 13 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-08758 BIOTECHDS

TITLE: Extending primer or pair of primers using enzymatic cycle primer extension reaction at lower temperatures, involves use of moderately thermostable DNA polymerase in combination with low concentration of glycerol;
DNA primer and DNA-polymerase for DNA sequencing

AUTHOR: HONG G F; YANG Y; ZHU J
PATENT ASSIGNEE: SHANGHAI MENDEL DNA CENT CO LTD

PATENT INFO: WO 2002101004 19 Dec 2002
APPLICATION INFO: WO 2002-IB3341 5 Jun 2002
PRIORITY INFO: US 2001-878131 8 Jun 2001; US 2001-878131 8 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-148788 [14]
AN 2003-08758 BIOTECHDS

AB DERWENT ABSTRACT:
NOVELTY - Extending primer or pair of primers (I) using an enzymatic cycle primer extension reaction below 80degreesC, is new.

DETAILED DESCRIPTION - Extending (M1) a primer or a pair of primers (I) using an enzymatic cycle primer extension reaction at temperatures below 80degreesC, involves mixing a template DNA with (I) and a natural or modified form of a moderately thermostable DNA polymerase (DP) from Bacillus stearothermophilus, B.caldotenax or B.caldolyticus, or is in solution containing 10-20% (volume/volume) (v/v) glycerol, ethylene glycol or its mixture, under conditions that cycle reaction temperature fluctuates between melting temperature of 70degreesC and annealing temperature of 37degreesC, so that DP repeatedly extends (I). An INDEPENDENT CLAIM is also included for a dry or liquid ready-to-use reaction mixture (II) suitable for use in low-temperature cycle primer extension reaction at a temperature below 80degreesC, comprising a moderately thermostable, natural or modified DNA polymerase from B.stearothermophilus, B.caldotenax or B.caldolyticus, that is pre-mixed with an enzymatic DNA primer extension reaction component suitable for use in DNA amplification or for specific extension terminations with dideoxyribonucleotide analogs.

BIOTECHNOLOGY - Preferred Method: The glycerol, ethylene glycol or its mixture is present in 15% (v/v), and DP has an optimum enzymatic activity at 65degreesC, and has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from B.stearothermophilus, B.caldotenax or B.caldolyticus. (M1) further involves repeating the cycle primer extension reaction. The copies of a selected segment of a **double-stranded DNA** are amplified in the presence of a **forward primer** and a **reverse primer** to the template by repeated heating and cooling cycles. The forward and reverse primers may be of varying lengths. Preferably in (M1), molecules of a single primer of various lengths are extended with specific nucleotide terminations in the presence of ddNTPs or their analogs for cycle sequencing. Preferred Reaction Mixture: (II) is pre-distributed into microcentrifuging tubes or in multiple-well plates, and remains stable at 22-25degreesC for at least 8 weeks. The moderately-stable DP is natural or modified DP from the above mentioned organisms, or a moderately thermostable DP which has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from B.stearothermophilus, B.caldotenax or B.caldolyticus.

USE - For extending primer or pair of primers (I) using enzymatic cycle primer extension reaction below 80degreesC. (M1) is useful for extending the molecules of a primer annealed to a DNA template for direct cycle sequencing of in vitro amplified **double-stranded DNA** products without prior isolation or purification, which involves mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard dideoxynucleoside triphosphate (ddNTP) terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase chosen from B.stearothermophilus, B.caldotenax or B.caldolyticus, a suitable

concentration of dNTP, and a composition comprising a buffer in a solution containing 10%-20% of glycerol, ethylene glycol or their mixture, and effecting cycle primer extension reaction(s) at a temperature below 80degreesC for sufficient number of times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs. The in vitro **double-stranded DNA** products employed in the method are generated by (M1) (all claimed).

ADVANTAGE - At the above mentioned concentration range, glycerol and/or ethylene glycol provide reduced melting temperature of the DNA template, and also increased the polymerization activity of the moderately thermostable DP. The methods use highly progressive moderately thermostable DP from B.stearothermophilus, B.caldotenax or B.caldolyticus. These polymerases have an optimum reaction temperature at 65degreesC, but are rapidly inactivated above 70degreesC, thus are useful as polymerizing enzymes for cycle primer extension to over some of the shortcomings of heat-resistant DNA polymerase.

EXAMPLE - Effect of glycerol on 5'-3' polymerization activity of moderately thermostable DNA polymerases was tested as follows. The remaining activity of Bst-II DNA polymerase (produced according US 6165765) in the presence of different concentrations of glycerol was determined as follows. In a series of 7 0.5 ml microcentrifuge tubes were added, 5 microl of 5X reaction buffer, 1 microl of 1 mM dNTPs and 1 microl of calf thymus DNA. The mixture above was firstly evaporated by Speed-Vacuum, then varying final concentrations of glycerol 0%, 10%, 13%, 20%, 30%, 40%, 50% in each reaction mixture were achieved by adding an appropriate amount of glycerol stock solution to the above microcentrifuge tubes. All these tubes were incubated at 65degreesC for 30 min. Then each of the reaction mixtures was pipetted onto a DE-81 filter. After all of the fluid had evaporated, the amount of radioactivity on each filter was measured with scintillation and recorded as X1. Thereafter, the filters were washed three times with 0.3 M Na2HPO4 solution at room temperature, 10 minutes each time, dried at room temperature and then the amount of radioactivity on each filter was measured again and recorded as X2. The incorporation ratio was X2/X1. The remaining activity (%) was calculated. Results showed that a low concentration of glycerol that did not exceed 20% (v/v) increased the enzymatic activity of Bst-II DNA polymerase. However, at higher concentrations glycerol exhibited an inhibitory effect on the enzyme. (61 pages)

L5 ANSWER 4 OF 13 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-03903 BIOTECHDS
TITLE: Amplifying specific nucleic acid molecules, useful for genotyping, allotyping, detecting genetic or infectious diseases, specific point mutations, or polymorphism, comprises providing a closed circular padlock probe molecule; DNA detection, DNA probe and DNA primer useful for genomic and disease diagnosis
AUTHOR: THOMAS D C
PATENT ASSIGNEE: VIRCO CENT VIROLOGICAL LAB IRELAND LTD
PATENT INFO: WO 2002068683 6 Sep 2002
APPLICATION INFO: WO 2002-EP2287 27 Feb 2002
PRIORITY INFO: US 2001-271433 27 Feb 2001; US 2001-271433 27 Feb 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-707011 [76]
AN 2003-03903 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A nucleic acid amplification method comprising providing a closed circular padlock probe molecule, a target nucleic acid molecule, a **forward primer**, a **reverse primer**, dNTPs, and a first DNA polymerase to form a reaction mixture, is new.
DETAILED DESCRIPTION - A nucleic acid amplification method

comprising: (a) providing a closed circular padlock probe molecule, a target nucleic acid molecule, a **forward primer**, a **reverse primer**, dNTPs, and a first DNA polymerase to form a reaction mixture; (b) creating a multi-tailed complex; (c) activating a second DNA polymerase, which is thermostable; and (d) thermocycling the multi-tailed complex. INDEPENDENT CLAIMS are also included for the following: (1) a nucleic acid molecule amplification kit comprising: (a) a **forward primer** and a **reverse primer**; (b) a ligase enzyme; (c) a first polymerase enzyme; (d) a linear padlock probe molecule comprising a 3' terminal region, a 5' terminal region, and a spacer region, which contains binding sites for the **forward primer** and the **reverse primer**; and (e) a second polymerase enzyme that is different from the first polymerase enzyme and is a thermostable enzyme; (2) detecting a target nucleic acid molecule or several target nucleic acid molecules in a sample; (3) a closed tube nucleic acid molecule amplification method comprising: (a) providing a target nucleic acid molecule, a ligase enzyme, a **forward primer**, a **reverse primer**, dNTPs, and a first DNA polymerase in a reaction tube; (b) sealing the reaction tube; (c) creating a closed circular padlock probe molecule; (d) creating a multi-tailed complex from the closed circular padlock probe molecule; (e) activating a second DNA polymerase, which is a thermostable DNA polymerase; and (f) thermocycling the multi-tailed complex; and (4) a padlock probe amplification primer comprises: (a) a sequence (S1); or (b) a sequence of 15-75 nucleotides; where at least one nucleotide base contained in the primer is a non-informative base analog. (S1) is 5'-actagagctgagaca-3'; 5'-actagagttcagaca-3'; 5'-actagagctgagacatgacga-3'; 5'-actagagttcagacatgacga-3'; 5'-actagagctgagacatgacgagtc-3'; 5'-actagagttcagacatgacgagtc-3'; 5'-actagagctgagacatgacgagtcgca-3'; or 5'-actagagttcagacatgacgagtcgca-3'.

BIOTECHNOLOGY - Preferred Method: The first DNA polymerase is Bst LF. The second DNA polymerase is a polymerase enzyme derived from Thermus aquaticus, Pyrococcus species, Thermococcus litoralis or Pyrococcus furiosus. The second DNA polymerase is a recombinant thermostable polymerase, which is an antibody-inactivated polymerase or a chemically inactivated polymerase. The reaction mixture further contains a strand displacement factor, where the reaction mixture is incubated for 1-30 minutes or 3-20 minutes at 50-70 degrees C prior to thermocycling. The nucleic acid amplification method further comprises denaturing the first DNA polymerase. The target nucleic acid molecule is: (a) a single-stranded DNA or RNA molecule; (b) a **double-stranded DNA** or RNA molecule; (c) a plasmid or its fragment; (d) a genomic DNA or its fragment; (e) a viral DNA or its fragment; (f) a viral RNA or its fragment; (g) a mRNA; (h) a mitochondrial DNA or its fragment; or (i) a chromosomal DNA or its fragment. The 5' terminal region of the forward or **reverse primer** is a tailed primer molecule. At least one of the **forward primer** or **reverse primer** is: (a) a hairpin primer that has a loop region containing a restriction endonuclease cleavage site; or (b) detectably labeled that contains a molecular energy transfer mechanism. At least one of the **forward primer** or **reverse primer** contains at least one polarity switch, or at least one non-informative base analog, preferably nitropyrrole or nitroindole. Detecting a target nucleic acid molecule in a sample comprises: (a) providing a target nucleic acid molecule, a linear padlock probe molecule, a ligase enzyme, a **forward primer**, a **reverse primer**, dNTPs, and a first DNA polymerase; (b) creating a closed circular padlock probe molecule; (c) creating a multi-tailed complex from the closed circular padlock probe molecule; (d) activating a second DNA polymerase; (e) thermocycling the multi-tailed complex with the second DNA polymerase; and (f) detecting the amplification product of the multi-tailed complex. Alternatively, the method comprises: (a) providing

a target nucleic acid molecule, a closed circular padlock probe molecule topologically linked to the target nucleic acid molecule, a **forward primer**, a **reverse primer**, dNTPs, and a first DNA polymerase; (b) creating a multi-tailed complex from the closed circular padlock probe molecule; (c) activating a second DNA polymerase; (d) thermocycling the multi-tailed complex with the second DNA polymerase; and (e) detecting the amplification product of the multi-tailed complex. Detecting several target nucleic acid molecules in a sample comprises: (a) providing several target nucleic acid molecules, several linear padlock probe molecules capable of annealing to several distinct target nucleic acid molecules, a ligase enzyme, dNTPs, and a first DNA polymerase; (b) creating at least two closed circular nucleic acid molecules, where each of the closed circular nucleic acid molecule is topologically linked to a distinct target nucleic acid molecule; (c) providing for each member of the two closed circular nucleic acid molecules, a **forward primer** and a **reverse primer**; (d) creating a multi-tailed complex for each of the distinct target nucleic acid molecules; (e) activating a second DNA polymerase; (f) thermocycling at least two distinct multi-tailed complexes with the second DNA polymerase; and (g) detecting the amplification products of the multi-tailed complexes. A real-time detection instrument performs the detection of the amplification products of the multi-tailed complexes. At least one of the two closed circular nucleic acid molecules, where the 5' terminal region of the forward or **reverse primer** does not hybridize to the closed circular nucleic acid molecule. The ligase enzyme used in the closed tube nucleic acid molecule amplification method, is a thermolabile ligase enzyme. At least one dNTP is a caged dNTP. Preferred Primer: The non-informative base analog is nitropyrrole or nitoindole, or a polarity switch.

USE - The methods are useful for detecting and amplifying specific nucleic acid molecules from a sample used for genotyping, pharmogenetics, detecting specific point mutations, detecting polymorphism, detecting rare events such as in cancer cells, allotyping, detecting genetic diseases, detecting aneuploidy, detecting microsatellite changes or detecting microbes or nucleic acid containing compositions. The methods are also useful in detecting infectious diseases caused by bacterial, viral, parasitic or fungal agents. The resistance of various infectious agents to drugs can also be determined using the methods. The methods can also be used in forensic areas such as for human identification for military personnel and criminal investigation, paternity testing and family relation analysis, human leukocyte antigen (HLA) compatibility typing, or screening blood, sperm or transplantation organs for contamination. The methods can also be used for identification and characterization of production organisms like yeast for the production of beer, wine or cheese.

ADVANTAGE - The present method is an improvement of the existing methods for amplifying padlock probes by eliminating or delaying the appearance of artifact products that can cause false positive results and improving the detectability of the probes, and also increases the sensitivity and speed of the assay.

EXAMPLE - No relevant example given. (51 pages)

L5 ANSWER 5 OF 13 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-11886 BIOTECHDS
TITLE: Diagnosing human hereditary hemochromatosis gene mutation of an individual, by performing single-tube high-throughput polymerase reaction assay for detection of mutation in the gene at position 845;
high throughput screening, polymerase chain reaction and DNA primer for disease diagnosis
AUTHOR: KAIRISTO V; DONOHOE G; ESKOLA J; KORPELA T
PATENT ASSIGNEE: KAIRISTO V; DONOHOE G; ESKOLA J; KORPELA T
PATENT INFO: WO 2002016637 28 Feb 2002

APPLICATION INFO: WO 2000-FI733 21 Aug 2000
PRIORITY INFO: FI 2000-1839 21 Aug 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-292067 [33]
AN 2002-11886 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Diagnosing (M1) human hereditary hemochromatosis gene (HFE) mutation of an individual, comprising performing single-tube high-throughput polymerase chain reaction (PCR) assay for detection of mutation in the gene at position 845 (C282Y), is new.

DETAILED DESCRIPTION - Diagnosing (M1) human hereditary hemochromatosis gene (HFE) mutation of an individual, comprising : (a) investigating DNA sample from an individual for the presence or absence of a mutation in the HFE gene at position 845; (b) applying covalently non-labeled oligonucleotide primers flanking to HFE gene position 845, where one of the primers amplifies healthy genomic DNA and another amplifies mutated genomic DNA; (c) adjusting the binding strength of the modified oligonucleotide primers by optimizing the nucleotide sequence near the 3'-end of the primers by deliberate base mismatches, adding a 5-50 bases long CG-tail to the 5-end of the primers; or adjusting the composition or concentration of ions in the solution of the polymerase chain reaction, in a way that the gene products, after subjecting genomic DNA to gene amplification process, deviates as regards to their melting temperatures and/or degree of amplification, effecting that the amplicons can be analyzed.

BIOTECHNOLOGY - Preferred Method: In M1, the primers preferably contain sequences having nucleotides that does not pair with the subject DNA, and the melting temperatures and amplification temperatures are alternatively amplicon lengths and amplification degrees, of each possible gene products are adjusted by a proper design of non-pairing nucleotides within the primers, where the primers are characterized by: 3' end of an oligonucleotide lies in the close vicinity of the position 845 of HFE gene, use of one **forward primer** including the sequence (S1) (nnatccaggcctgggtgctccacctnny) for amplification of normal DNA and one **forward primer** including (S1) for amplification of mutant DNA with one **reverse primer** amplifying both alleles or alternatively, the use of primers except that of one **forward primer** additionally contains in its 5'end additional priming or non-pairing oligonucleotide tail any of the oligonucleotides do not hybridize with the intron sequences of the HFE gene. In M1, the amplification is performed by techniques known in prior art while the amplification products are detected by electrophoretic method or by methods able to differentiate the melting temperatures of the gene products, and after a single amplification reaction, the gene products can be analyzed and the results show whether the gene material is healthy, homo- or heterozygous as to the single nucleotide polymorphism of the HFE gene. The oligonucleotide primers are exploited with a DNA to be diagnosed in a single-tube reaction with a fluorescent dye, preferably SYBR Green I, or related dye able to monitor the presence or absence of **double-stranded DNA**. The reaction mixture is subjected to a DNA-amplification procedure while the fluorescence of the monitoring dye is followed continuously or analyzed after completion of the amplification reaction. The judgment of possible abnormalities in HFE gene is based on the existence of the position and number of peaks in the obtained DNA melting curve. Derivative fluorescence melting curves of healthy individuals, as to hereditary hemochromatosis, show one peak as well as the patients of the disease under consideration, but the peaks appear at distinct melting temperatures, carriers of hereditary hemochromatosis show two peaks of derivative fluorescence melting curves. One peak corresponding to normal and the other one to patient of hereditary hemochromatosis. The analysis reaction is performed in a single vessel and the result of the analysis shows whether the studied DNA belongs to a healthy, homo-heterozygotic

individual or hereditary hemochromatosis, and with a DNA sample to be diagnosed. The reaction mixture with due amplification reagents which are subjected to a DNA amplification process in a single, preferably sealed reaction vessel. After the amplification process, the reaction products are analyzed with by electrophoretic techniques known in prior art, revealing the sizes of specific amplicons, followed by their analysis based on their location compared with standard DNA markers. The bands on the electrophoresis support show whether the sample belongs to a healthy, homo- or heterozygotic individual in respect to hereditary genetic hemochromatosis.

USE - M1 is useful for diagnosing HFE mutation of an individual (claimed).

ADVANTAGE - The design of specific mismatch primers enables to detect both normal and mutated alleles in one PCR reaction. The reaction mixture does not necessarily need to be subjected to any further analysis. The developed method is more reliable and more economical than described in the prior art. The assay can be carried out without opening the reaction vessel since the amplification products can be analyzed through the transparent or opalescent tubes. This is a very remarkable advantage because PCR diagnostic laboratories tend to be contaminated readily by the reaction products. However, the PCR products can be also subjected to traditional detection such as electrophoresis on agarose gel.

EXAMPLE - More than 200 subjects for the C282Y mutation were tested. The DNA extraction was carried out using the salting-out principle. The genome DNA was amplified by polymerase chain reaction (PCR) using primers: a wild type primer HFEW (5'-GGGGGGCCCCGGGCCCAGATCACAATGAGGGGCACA TCCAGGCCTGGGTGCTCCACCTCGC-3' and a mutant primer HFEM (5'-TGATCCAGGCCTGGGTGCTCCACCTGCT-3' and **reverse primer** that amplified both alleles, a common primer HFECOM (5'-CAGGGCTGGATAACCTTGGCTGTACC-3'), and a fluorescent dye SYBR Green I, that can detect **double-stranded DNA** (**dsDNA**). Negative control reactions containing water in place of DNA were included in each batch of PCR reactions to exclude appearance of contamination. The PCR products were analyzed on gel, where the amplicons were sized using a 50 base pair molecular mass marker. Mispriming and cross reactions were prevented by the introduction of deliberate mismatches between primers and template. The results showed that for each C282Y sample the allele specific primers accurately distinguished between mutant homozygote, wildtype and heterozygote. The melting of the sample homozygous for the 845 G showed a marked change in fluorescence between 85 degrees C and 87 degrees C, with a clear maximum rate of change at 86 degrees C. In contrast, the sample homozygous for the 845 A allele, showed a marked decrease in fluorescence between 82-84 degrees C, with a clear maximum rate of change at 83 degrees C. The heterozygous sample contained both fluorescent melting peaks due to the presence of amplicons derived from both alleles. Analysis of the products by standard slab electrophoresis revealed that the GC-tailed primer HFEW2 was specific for the wild-type G allele whereas the short primer HFEM was specific for the A allele.(32 pages)

L5 ANSWER 6 OF 13 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-02555 BIOTECHDS
TITLE: Novel oligonucleotide for detecting Salmonella toxin gene invA or stn mRNA, capable of specifically binding to Salmonella toxin gene invA or stn mRNA; DNA probe or DNA primer for gene detection and bacterium analysis
AUTHOR: YOKOYAMA A; ISHIGURO T
PATENT ASSIGNEE: TOSOH CORP
PATENT INFO: EP 1233073 21 Aug 2002
APPLICATION INFO: EP 2002-1254 17 Jan 2002
PRIORITY INFO: JP 2001-9464 17 Jan 2001; JP 2001-9464 17 Jan 2001
DOCUMENT TYPE: Patent

LANGUAGE: English
OTHER SOURCE: WPI: 2002-668421 [72]
AN 2003-02555 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An oligonucleotide (I) for detecting Salmonella toxin gene invA or stn mRNA, that is capable of specifically binding to Salmonella toxin gene invA or stn mRNA, is new.

DETAILED DESCRIPTION - (I) that specifically binds to Salmonella toxin gene invA mRNA, comprises 10 contiguous bases of a sequence chosen from 12 sequences (S1-S12) fully defined in the specification such as AGACGACTGGTACTGATCGA, AGGAACCGTAAAGCTGGCTT, TAATGATGCCGGCAATAGCG, ATCAACAATGCGGGGATCTG, ATTTACGCGGGTCACGATAA, and CTGCGTCATGATATTCCGCC; or that binds to Salmonella toxin gene stn mRNA, comprises 10 contiguous bases of the sequence (S13-S18) AAGGTGAAAAGTATTGAGGG, GATAGCGGGAAAGGGATCGC, AGGCTGACTCAGGTGCTGTT, ATATTATTACTCACTCCCTG, GGGGCATCTGGCGGCGGGCG, or ATGAAGCGTAAAGAAAAGCT. INDEPENDENT CLAIMS are also included for the following: (1) amplifying Salmonella gene invA or stn nRNA, where a specific sequence of Salmonella gene invA or stn mRNA present in a sample is used as a template for synthesis of a cDNA employing an RNA-dependent DNA polymerase, the RNA of the formed RNA/DNA hybrid is digested by ribonuclease H to produce a single-stranded DNA which is then used as a template for producing a **double-stranded DNA** having a promoter sequence capable of transcribing RNA comprising the specific sequence or a sequence complementary to the specific sequence employing a DNA-dependent DNA polymerase, where **double-stranded DNA** in the presence of an RNA polymerase, produces an RNA transcription product which is then used as a template for cDNA synthesis employing the RNA-dependent DNA polymerase, involves: employing (I) and another oligonucleotide (II) comprising 10 contiguous bases of the sequence chosen from 5 sequences given in the specification such as AATTCTAATACGACTCACTATAGGGAGATTCCTTTGACGGTGCGATGA or AATTCTAATACGACTCACTATAGGGAGAGGCATCATTATTATCTTTGT; or sequences, e.g. AATTCTAATACGACTCACTATAGGGAGAACCTTAATCGCGCCGCCATG and AATTCTAATACGACTCACTATAGGGAGACTATCGGTAACAGTGATGAT, respectively, and having a sequence homologous to a portion of the Salmonella gene invA or stn mRNA sequence to be amplified, where the first or second oligonucleotide includes the RNA polymerase promoter sequence at the 5' end; and (2) a kit comprising (I) and/or (II), and optionally, reagents for the transcription of RNA into cDNA or amplification of nucleic acid molecules.

BIOTECHNOLOGY - Preferred Method: The amplification process is a detection method, which is carried out in the presence of an oligonucleotide probe capable of specifically binding to the RNA transcription product resulting from the amplification and labeled with an intercalator fluorescent pigment. The changes in the fluorescent properties of the reaction solution is measured, with the proviso that the labeled oligonucleotide has a sequence different from those of (I) and (II). The probe is designed to complementarily bind to a portion of the sequence of the RNA transcription product, and the fluorescent property changes relative to that of a situation, where a complex formation is absent. The probe for detecting the invA or stn mRNA comprises 10 contiguous bases of the sequence TCAGCATGGTATAAGTAGACAGGGCG (its complement) or AGCGTAGAGGCAAAAGAAAGTGGGAC (its complement), respectively.

USE - (I) is useful for detecting Salmonella toxin gene invA or stn mRNA (claimed). (I) is useful as primers for amplifying the target RNA (Salmonella toxin gene invA or stn mRNA or their complementary sequence) by nucleic acid sequence based amplification (NASBA) or 3SR at 30-50degreesC and at a constant temperature (41degreesC).

ADVANTAGE - (I) allows simple, rapid and highly sensitive amplification and detection of target RNA. (I) achieves the effect of making unnecessary denaturation of RNA, prior to the amplification process.

EXAMPLE - Selection of oligonucleotide which specifically bound to Salmonella toxin gene invA was selected as follows. Polymerase chain reaction (PCR) was carried out on the region of base Nos. 104-2052 of the base sequence of Salmonella toxin gene invA using a **forward primer** to which the promoter sequence of T7 RNA polymerase was added at the 5' end and having a sequence homologous to the base Nos. 104-122, and a **reverse primer** having a sequence complementary to the base Nos. 2029-2052. The above PCR product was used as template in preparing a standard RNA by a transcription reaction. The PCR-produced template was then digested with a DNA polymerase, and the standard RNA was purified. The standard RNA was quantified by ultraviolet absorption at 260 nm, and then diluted, with an RNA diluent. The reaction solutions (containing Tris HCl buffer pH 7.5, potassium chloride, magnesium chloride, dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA), standard RNA, oligonucleotide (chosen from 12 sequences (S1-S12) such as AGGAACCGTAAAGCTGGCTT, TAATGATGCCGGCAATAGCG and ATCAACAATGCGGGGATCTG)) were then incubated at 41degreesC for 5 min, and then 1 microl of 0.1 U/microl RNase H was added. The PCR tubes were incubated at 41degreesC for 15 min. Urea-modified polyacrylamide gel electrophoresis was conducted to confirm the cleaved fragments following the reaction, and then dyeing was carried out. When the oligonucleotide specifically bound to the standard RNA, the standard RNA was digested in this binding region to yield a digestion product having a characteristic chain length. Cleavages at expected positions were confirmed for oligonucleotides S1-S12. It was demonstrated that the oligonucleotides firmly bound to Salmonella toxin gene invA mRNA at 41degreesC and in a constant state. Detection in a various number of initial copies of Salmonella toxin gene invA mRNA was carried out using combinations of oligonucleotides as follows. The standard RNA was diluted to concentrations ranging from 105-102 copies/5 microl with an RNA diluent. In the control, only the diluent was used. A reaction solution containing Tris-HCl buffer, MgCl2, KCl, RNase inhibitor, DTT, dATP, dCTP, dGTP, dTTP, ITP, ATP, CTP, GTP, UTP, first oligonucleotide (TAATGATGCCGGCAATAGCG), second (AATTCTAATACGACTCACTATAGGGAGACATTATTATCTTTGTGAACT), third (ATCAACAATGCGGGGATCTG), intercalator fluorescent pigment-labeled oligonucleotide (TCAGCATGGTATAAGTAGACAGGGCG) and dimethylsulfoxide (DMSO), was dispensed into PCR tubes. The RNA sample in an amount of 5 microl was added. After incubating the reaction solution for 5 min at 41degreesC, 4.2 microl of an enzyme solution having sorbitol 1.7%, bovine serum albumin (3 mug), T7 RNA polymerase (142 U), and AMV-reverse transcriptase (8 U) was added. The PCR tube was then incubated at 41degreesC using a direct-measurable fluorescence spectrophotometer equipped with a temperature controller. The initial RNA amount was between 101-105 copies/30 microl. 102 copies were detected in 13 min. A fluorescent profile and a calibration curve both depending on the initial concentration of the labeled RNA was obtained, which indicated that it was possible to quantify the target RNA present in unknown samples, and demonstrated that the detection of invA mRNA was rapid and highly sensitive. (28 pages)

L5 ANSWER 7 OF 13 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-10070 BIOTECHDS
TITLE: Determining whether or not continued known DNA sequence in genome of biological species (BS) is gene expression region, by detecting in RNA of BS, a nucleotide sequence (NS) corresponding to NS of specific region;
recombinant methanol-assimilating yeast, genome analysis, DNA amplification and RNA amplification for gene detection
AUTHOR: ISHIGURO T; YASUKAWA K
PATENT ASSIGNEE: TOSOH CORP
PATENT INFO: EP 1174521 23 Jan 2002
APPLICATION INFO: EP 2000-116674 14 Jul 2000
PRIORITY INFO: JP 2000-334935 30 Oct 2000
DOCUMENT TYPE: Patent

LANGUAGE: English
OTHER SOURCE: WPI: 2002-156698 [21]
AN 2002-10070 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Determining (M1) whether or not continued arbitrary DNA sequence existing in the genome of arbitrary biological species (BS) is the specific region, where the nucleotide sequence (NS) is known but its possibility of being gene expression region (specific region) is unclear, involves detecting whether or not NS that corresponds to the NS of the region is present in the RNA of BS.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a method for determining the gene expression region in an arbitary region on a genome or the entire genomes, comprises repeatedly carrying out M1; (2) a genomic gene which was determined to be gene expression region by repeatedly carrying out (M1); and (3) a protein encoded by the above described gene.

BIOTECHNOLOGY - Preferred Method: The specific region is a DNA region of 100-200 bases, The detection step is carried out by detecting whether or not DNA or RNA is amplified by carrying out amplification of DNA or RNA based on the RNA of the BS, using an oligonucleotide homologous to a sequence which comprises 10 or more contiguous bases and is positioned in the 5' end of the specific region and another oligonucleotide complementary to a sequence which is comprised of at least 10 or more contiguous bases and positioned in the 3' end of the specific region. At least one of the oligonucleotides used in the method has a RNA-transcriptable promoter sequence in its 5' end and amplification is an RNA amplification which involves: (1) synthesizing a DNA fragment complementary to a part of RNA of the BS by RNA-dependent DNA polymerase from either one of the oligonucleotides using BS-derived RNA as the template, thereby effecting formation of an RNA-DNA hybrid; (2) forming a single-stranded DNA fragment by hydrolyzing the BS-derived RNA of the RNA-DNA hybrid with ribonuclease H; (3) synthesizing a DNA fragment complementary to the single-stranded DNA fragment by DNA-dependent DNA polymerase from the other oligonucleotide using the single-stranded DNA fragment as the template, thereby effecting formation of a **double-stranded DNA** fragment having a promoter sequence capable of performing transcription of RNA as a part of the RNA of the BS or RNA complementary to it; (4) forming RNA transcription product from the **double-stranded DNA** using RNA polymerase; and (5) repeating the all the above steps using the RNA transcription product as the template. Preferably, detecting whether or not DNA or RNA is amplified is carried out by carrying out the amplification in the presence of an oligonucleotide probe which can specifically bind to the DNA or RNA formed by the amplification and is labeled with an interacting fluorescence dye, provided that the oligonucleotide is a sequence which does not form complementary bonding with any one of the above mentioned oligonucleotides, and measuring the change in a fluorescence characteristic of the reaction solution. The probe is capable of performing complementary binding with at least a part of the sequence of the DNA or RNA transcription product formed by the amplification to change the fluorescence characteristic as compared with the case in which the complex is not formed.

USE - For determining whether or not a continued arbitrary sequence existing in the genome of an arbitrary BS is the specific region, where the nucleotide sequence is unknown but its possibility of being a gene expression region is unclear. The method is repeatedly carried out for determining gene expression region in arbitrary region on genome or the entire genome (claimed).

ADVANTAGE - The determination can be effected within a short period of time because the amplification is completed within a markedly short time of 10 minutes. The method has high sensitivity, which enables amplification of even several pg of RNA containing the specific region, and also the possibility of contamination of RNA by DNA is excluded.

EXAMPLE - Establishment of gene expression region was carried out as follows. As the genomic region, a region composed of 900 base pairs prepared from a G1 strain, a genetically engineered transformed methanol assimilating yeast strain was selected. When induced by methanol, the G1 strain expressed a human interleukin(IL)-6R-IL-6 fusion protein composed of one polypeptide chain of 397 amino acid residues. The region composed of 900 base pairs was divided into five specific regions each having 180 base pairs. The mRNA expressing mode of the region was also already known. The specific region 1 (base numbers 1 to 180) contained 159 base pairs of a non-transcription region and 21 base pairs of a transcription region. Each of the specific region 2 (base numbers 181 to 360), specific region 3 (base numbers 361 to 540), specific region 4 (base numbers 541 to 720) and specific region 5 (base numbers 721 to 900) contained only a transcription region. Oligonucleotide (primer) sets (**forward primer** F (1F-5F), **reverse primer** R (1R-5R) and scissor probe S (1S-5S)) were synthesized for each of the above five specific regions, that have specific sequences as given in specification. RNA amplification (TRC; transcription reverse transcription concerting amplification) was carried out using forward and reverse primers and scissor probe. In the TRC, a specific region cannot be amplified when it was not located at the 5'-terminal of mRNA. The scissor probe was an oligonucleotide (DNA) to be used in the case for locating the specific region of the 5'-side of mRNA by complementarily binding it to the 5'-side of the specific region and cutting the complementarily bounded region by the action of a ribonuclease. An mRNA sample of the strain G1 was prepared by the following method. The strain was inoculated into 3 ml of BMGY (Bacto Yeast Extract 10 g/l, Bacto Peptone 20 g/l, Yeast Nitrogen Base without amino acids 1.34 g/l, 100 mM potassium phosphate buffer, pH 6.0, glycerol 10 g/l and biotin 0.4 mg/l) medium, and cultured at 28 degrees Centigrade for 24 hours. A 100 microlitres portion of the culture broth was inoculated into 3 ml of BMGY (Bacto Yeast Extract 15 g/l, Bacto Peptone 30 g/l and other components having the same composition of the above BMGY) medium, and cultured at 28 degrees Centigrade for 16 hours. After confirmation of the depletion of methanol, 100 microlitres of methanol was added to the medium to induce expression of the human interleukin (IL)-6R-IL-6 fusion protein. Two hours after the addition of methanol, the cells were collected and subjected to cell wall lysis. Next, mRNA was prepared. Determination of gene expression region by RNA amplification was carried out as follows. The mRNA was diluted to 200 ng/5 microlitres. A 20.8 microlitres portion of a reaction solution comprising 0.16 microMolar of scissor probe, 1 microMolar of **forward primer**, 1 microMolar of **reverse primer** was added with 5 microlitres of the above RNA sample. This reaction solution was incubated at 65 degrees Centigrade for 15 minutes and then at 41 degrees Centigrade for 5 minutes, and then 4.2 microlitres of an enzyme solution comprising 1.7% of sorbitol, 3 micrograms of bovine serum albumin, 142 U of T7 RNA polymerase, 8 U of AMV reverse transcriptase was added. Subsequently, the tubes were kept at 41 degrees Centigrade for 10, 20 or 30 minutes. Immediately after the reaction, an electrophoresis was carried out. Amplification was not found when the primer for the specific region 1 was used in each case of the 10 minute reaction, 20 minute reaction and 30 minute reaction but was found when the primers for the specific regions 2 to 5 were used. These results showed that the RNA amplification was specific for a primer derived from a region composed solely of a gene expression region, i.e., whether or not a continued arbitrary DNA sequence existing in the genome of an arbitrary biological species, in which the nucleotide sequence was already known but its possibility of being a gene expression region was unclear (specific region), was a gene expression region can be determined by detecting the presence or absence of a nucleotide sequence which correspond to the nucleotide sequence of the region in the RNA of the biological species, by an RNA amplification typified by TRC. (22 pages)

L5 ANSWER 8 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2002:334489 CAPLUS
DOCUMENT NUMBER: 137:333615
TITLE: Quantitative detection of Neospora caninum in bovine aborted fetuses and experimentally infected mice by real-time PCR
AUTHOR(S): Collantes-Fernandez, Esther; Zaballos, Angel; Alvarez-Garcia, Gema; Ortega-Mora, Luis M.
CORPORATE SOURCE: Departamento de Sanidad Animal, Universidad Complutense de Madrid, Madrid, 28040, Spain
SOURCE: Journal of Clinical Microbiology (2002), 40(4), 1194-1198
CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We report the development of a real-time PCR assay for the quant. detection of Neospora caninum in infected host tissues. The assay uses the **double-stranded DNA**-binding dye SYBR Green I to continuously monitor product formation. Oligonucleotide primers were designed to amplify a 76-bp DNA fragment corresponding to the Nc5 sequence of N. caninum. A similar method was developed to quantify the 28S rRNA host gene in order to compare the parasite load of different samples and to correct for the presence of potential PCR-inhibiting compds. in the DNA samples. A linear quant. detection range of 6 logs with a calculated detection limit of 10-1 tachyzoite per assay was observed with excellent linearity (R2 = 0.998). Assay specificity was confirmed by using DNA from the closely related parasite Toxoplasma gondii. The applicability of the technique was successfully tested in a variety of host brain tissues: (i) aborted bovine fetuses classified into neg. or pos. Neospora-infected animals according to the observation of compatible lesions by histopathol. study and (ii) exptl. infected BALB/c mice, divided into three groups, inoculated animals with or without compatible lesions and neg. controls. All samples were also tested by ITS1 Neospora nested PCR and a high degree of agreement was shown between both PCR techniques (κ = 0.86). This technique represents a useful quant. diagnostic tool to be used in the study of the pathogenicity, immunoprophylaxis, and treatment of Neospora infection.
REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 13 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-04916 BIOTECHDS
TITLE: Transforming chromatographic elution profiles, useful for analyzing DNA polymorphisms or mutations, by subjecting profiles obtained by Denaturing Matched Ion Polynucleotide Chromatography to a computer implemented method; bioinformatics and transforming chromatographic elution profile useful for disease-resistant transgenic plant and single point mutation polymorphism detection
AUTHOR: TAYLOR P D; YU E
PATENT ASSIGNEE: TRANSGENOMIC INC
PATENT INFO: WO 2001095233 13 Dec 2001
APPLICATION INFO: WO 2000-US17949 2 Jun 2000
PRIORITY INFO: US 2001-282070 5 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-114469 [15]
AN 2002-04916 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Transforming chromatographic elution profiles, each of which is obtained from the separation of a DNA mixture by Denaturing Matched Ion Polynucleotide Chromatography, comprising employing a computer implemented method to analyze profiles obtained by Denaturing Matched Ion

Polynucleotide Chromatography, is new.

DETAILED DESCRIPTION - Transforming chromatographic elution profiles, each of which is obtained from the separation of a DNA mixture by Denaturing Matched Ion Polynucleotide Chromatography, comprising: (a) overlaying the profiles on a coordinate system comprising a first axis associated with time values and a second axis associated with detector response values; (b) selecting first and second time points defining a time span, where peaks due to the homoduplex and heteroduplex molecules are located within the span; (c) for each profile and within the span, adjusting the baseline by applying a slope factor to each detector response value, the factor derived from a line connecting the detector response values at the first and second time points, so that all of the profiles share a common baseline; (d) for each profile and within the span, normalizing the heights of the peaks to a pre-selected scale based on the height of the highest peak; and (e) shifting the profiles along the first axis such that all of the profiles intersect at a pre-selected point on the last eluting peak of each profile within the span.

INDEPENDENT CLAIMS are also included for the following: (1) estimating the number of different single nucleotide polymorphisms in several same length DNA fragments; (2) detecting the presence of a previously unknown single nucleotide polymorphism in a test DNA fragment; (3) a computer implemented method for grouping several transformed chromatographic elution profiles obtained by the novel method; (4) grouping several transformed chromatographic elution profiles obtained by the novel method; (5) a system for transforming chromatographic elution profiles comprising a computer having a processor and memory, where the computer receives a set of data corresponding to several chromatographic elution profiles, where each profile is obtained from the separation of a DNA mixture by Denaturing Matched Ion Polynucleotide Chromatography, where each DNA mixture comprises homoduplex and heteroduplex molecules obtained from the hybridization of a sample DNA and its corresponding wild type DNA, and where the processor performs the novel method; (6) computer readable media for storing computer readable instructions, the instructions being capable of programming a computer to perform the methods above; and (7) transformed elution profiles obtained, or elution profiles grouped by the novel method.

BIOTECHNOLOGY - Preferred Method: In transforming chromatographic elution profiles, the pre-selected value is zero, where the pre-selected scale is 0-1. The pre-selected point comprises a point on the last eluting edge of the last eluting peak. In step (c), the second axis value at the first time point and the second axis value at the second time point are set to zero. The profiles include at least one reference profile obtained from a standard mixture comprising the hybridization product of DNA having a known sequence and corresponding wild type DNA. The method of (1) comprises: (a) hybridizing each of the same length DNA fragments with corresponding wild type DNA to form homoduplex and heteroduplex molecules; (b) analyzing the hybridization product of each of the same length DNA fragments by Denaturing Matched Ion Polynucleotide Chromatography to obtain elution profiles; (c) transforming the profiles by employing the novel method; and (d) sorting the transformed profiles into groups based on the shapes of the transformed profiles, where the number of single nucleotide polymorphisms is at least the same as the number of the groups. In method (2), detecting the presence of a previously unknown single nucleotide polymorphism in a test DNA fragment comprises: (a) hybridizing the test DNA fragment with corresponding wild type DNA; (b) analyzing the product of step (a) by Denaturing Matched Ion Polynucleotide Chromatography to obtain a test elution profile; (c) hybridizing a standard mixture comprising DNA fragments of known sequence with the wild type DNA; (d) analyzing the product of step (c) by Denaturing Matched Ion Polynucleotide Chromatography to obtain reference elution profiles; (e) obtaining profiles by combining the test elution profiles and the reference elution profiles; (f) transforming the profiles by employing the novel method; (g) sorting the profiles into groups based on the shapes of the elution profiles; and (h) after the

transforming, comparing the test elution profile with the groups, where the test DNA fragment is considered to contain a previously unknown mutation if the shape of the test elution profile does not match the shapes of the profiles in the groups. The method further includes subjecting the test DNA fragment to sequencing. The method also includes applying one or more statistical criteria to the transformed profiles obtained after step (e) to determine whether or not to group the transformed profiles into a single group. In particular, the statistical criteria comprise: (a) within the span, dividing the first axis into a series of adjacent and evenly-spaced time regions where boundary lines, perpendicular to the first axis, are defined between adjacent regions, and where the profiles intersect the boundary lines at intersecting detector response values; and (b) for each boundary line: (i) obtaining the mean of the intersecting detector response values, and comparing the mean to a first pre-selected value; (ii) obtaining the standard deviation of the mean of the intersecting detector response values, and comparing the standard deviation to a second pre-selected value; and (iii) obtaining the range of the intersecting detector response values, and comparing the range to a third pre-selected value. The method of (3) comprises: (a) within the span, dividing the first axis into a series of adjacent and evenly-spaced time regions where boundary lines, perpendicular to the first axis, are located between adjacent time regions, where the profiles intersect the boundary lines; (b) for each boundary line and between the highest intersecting profile and the lowest intersecting profile, dividing the each boundary line into equally spaced and adjacent segments; (c) for each boundary line, numbered 1 through i: (i) determining the number of profiles intersecting each of the segments; (ii) determining the segment having the highest number of intersecting profiles and determining the nearest segment having zero intersecting profiles; and (iii) for each boundary line, assigning a numerical grouping factor of n-i to the profiles that have a second axis value greater than the segment having zero intersecting profiles and assigning a grouping factor of 1 to the remaining intersecting profiles, where n is an integer greater than 1; (d) for each profile, obtaining a total value comprising the sum of all the grouping factors assigned to the each profile; and (e) grouping together those profiles having the same total value. In particular, n = 2. In method (4), grouping transformed chromatographic elution profiles comprises: (a) placing one or more markers, numbered 1 through i, each marker placed at a position where the transformed elution profiles show apparently clustered detector response values; (b) obtaining the first axis value and second axis value for each marker, each marker located on a boundary line perpendicular to the first axis; (c) for each marker, and along its associated boundary line, assigning a numerical grouping factor of n-i to the profiles that have a second axis value greater than the second axis value of the each marker, or otherwise assigning a grouping factor of 1 to the profiles, where n is an integer greater than 1; (d) for each profile, obtaining a total value comprising the sum of all the grouping factors assigned to the each profile; and (e) grouping together those profiles having the same total value. Preferred System: The system further comprises a processor that receives instructions for employing the methods of (3) and (4).

USE - The computer implemented method is useful for estimating the number of different single nucleotide polymorphisms in several test samples and for detecting the presence of a previously unknown polymorphisms. The method is useful for detecting mutations in DNA and for analyzing elution profiles obtained from liquid chromatographic separation of **double-stranded DNA**.

Detection of mutations and identification of similarities or differences in DNA samples is of critical importance in increasing the world food supply by developing diseases, resistant and/or higher yielding crop strains, in forensic science, in the study of evolution and populations, and in scientific research in general.

ADVANTAGE - Analysis of DNA samples has historically been done using gel electrophoresis. Capillary electrophoresis has been used to separate

and analyze mixtures of DNA. However, these methods cannot distinguish point mutations from homoduplexes having the same base pair length. The present method is an accurate and reproducible analytical method for mutation detection, which is easy to implement. The present method can be automated and can provide high throughput sample screening with a minimum of operator attention.

EXAMPLE - Mutations in an amplicon from Homo sapiens haemochromatosis HFE gene was scanned. The amplicon covers nucleotides 6614-6771 (158 base pair product). Sample DNA was amplified by conventional touchdown polymerase chain reaction (PCR) methods using a proof-reading polymerase and using the following primers. **Forward primer**: 5'-TGGATGCCAAGGAGTTCGA **Reverse primer** : 5'-ACCCCAGATCACAATGAGGG After amplification, each sample was mixed with an equimolar amount of wild type **dsDNA** and hybridized. The mixture was separated using a WAVE (RTM) DNA Fragment Analysis System. Results showed 96 elution profiles selected from Denaturing Matched Ion Polynucleotide Chromatography analysis of 96 amplified DNA samples. A first group of elution profiles that was identified using the automated grouping method showed that the DNA in the samples in this first group was the wild type DNA. A second group that was identified using the automated grouping method showed that the DNA possessed the G-A mutation. Results showed that the mutation was a G-A mutation at position 6722 that gave rise to a C282Y mutation in the expressed protein. (77 pages)

L5 ANSWER 10 OF 13 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-07337 BIOTECHDS
TITLE: Detecting presence of polynucleotide, differences between polynucleotide sequences, useful for detecting single nucleotide polymorphism and alleles of polynucleotide sequence involves use of three competitive probes; the use of DNA probe and polymerase chain reaction useful for SNP detection
AUTHOR: PATEL R D
PATENT ASSIGNEE: DADE BEHRING INC
PATENT INFO: WO 2001090399 29 Nov 2001
APPLICATION INFO: WO 2000-US16089 19 May 2000
PRIORITY INFO: US 2000-574596 19 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-097664 [13]
AN 2002-07337 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Detecting presence of polynucleotide analyte (NA), or one or both first and second NA which differ from each other by one or more nucleotides (nt) or by insertion or deletion of nt, by using 3 competitive probes (P1-P3). P1 hybridizes to first region on NA. P2,P3 are capable of hybridizing to second region on NA. P2,P3 are identical except for presence of difference, deletion and insertion of one or more nt.

DETAILED DESCRIPTION - Detecting (M1) the presence of a NA involves: (a) combining NA and (P1)-(P3) in a reaction medium, P1 comprising a sequence complementary to a R1 of NA, P2 comprising a sequence complementary to region of NA, and P3 comprising a sequence complementary to R2 except for difference of at least one nucleotide, or the insertion or deletion of at least one nucleotide; (b) subjecting the reaction medium to conditions for forming substantially non-dissociative termolecular complexes (TC) of at least one of: (i) NA, P1 and P2, or (ii) NA, P1 and P3; (c) determining the presence of at least one of TC or (d) detecting (M2) the presence of one or both of a first and a second NAs which differ from each other by one or more nucleotides or by the insertion or deletion of at least one nucleotide, involves: (a) combining in a reaction medium a sample suspected of containing one or both NAs, and three oligonucleotide probes (OP1-OP3), where OP1 comprises a sequence which hybridizes to a sequence common to both the first and

second NAs, OP2 comprises a sequence which hybridizes to a second region of first NA, and OP3 comprises the same sequence as OP2 except for a difference of at least one nucleotide, or the insertion or deletion of at least one nucleotide, where the difference, the insertion or the deletion represents the difference, insertion or deletion between the first and second NAs; (b) subjecting a reaction medium to conditions forming at least one of: (i) first TC comprising NA, OP1 and OP2, (ii) a second TC comprising the second NA, OP1 and OP3; and (c) detecting the presence of at least one of the first and second TC. An INDEPENDENT CLAIM is also included for a kit (I) for use in a determination of a target polynucleotide in a sample, comprising: (a) a first oligonucleotide probe comprising a sequence which hybridizes to a first region of the target polynucleotide; (b) a second oligonucleotide probe which hybridizes to a second region of the target polynucleotide; (c) a third oligonucleotide probe comprising the same sequence as the sequence of the second probe except for the difference of one or more nucleotides or the insertion or deletion of one or more nucleotides, that is complementary to an expected mutation of the target polynucleotide; (d) a first label comprising a first member of first and second signal producing systems; (e) a second label comprising a second member of the first signal producing system; and (f) a third label comprising a second member of the second signal producing system; where the three labels non-covalently bind to the first, second, third oligonucleotide probes, respectively.

BIOTECHNOLOGY - Preferred Method: (M1) further comprises a first, second, third labels bound to P1, P2, P3, respectively. The first label comprises a first member of first and second signal producing systems, the second label comprises a second member of the first signal producing system, and the third label comprises second member of the second signal producing system. The labels are non covalently bound to the probes. The first and second members of the signal producing systems are any one of luminescent energy donor and acceptor pair, a fluorescence energy donor and acceptor pair, a singlet oxygen generator and chemiluminescent reactant pair, and an enzyme pair where a product of the first enzyme serves as a substrate for the second enzyme. Preferably, the first member of the signal producing systems is a sensitizer (e.g., a photosensitizer such as methylene blue, rose bengal, porphyrins and phthalocyanines or naptholcyanines) when the second members of the signal producing systems are chemiluminescent compounds (e.g., enol ethers, enamines, 9-alkylidene-N-alkylacridans, arylvinylethers, dioxenes, arylimidazoles, 9-alkylidene-xanthanes or lucigenin) or vice versa. (P1)-(P3) further comprise second sequences which do not hybridize to the NA or each other, the first label further comprises a sequence which hybridizes to the second sequence of P1, the second label comprises a sequence which hybridizes to the second sequence of P2, and the third label comprises a sequence which hybridizes to the second sequence of P3. The reaction medium further comprises reagents sufficient for amplifying the polynucleotide analyte, and prior to step (b), the medium is subjected to conditions for amplifying the polnucleotide probe. Preferred Kit: (I) further comprises reagents for conducting an amplification of the polynucleotide analyte.

USE - (M1) is useful for detecting the presence of a single nucleotide polymorphism (SNP) in a fragment of genomic DNA which involves combining fragment of genomic DNA suspected of containing SNP and (P1)-(P3), where P2 and P3 are identical except for the difference of one nucleotide, the difference being complementary to SNP in the second sequence; subjecting the combinations for annealing the first, and second and third probe to the analyte, where the interaction of first and second label bound to P1 and P2 produces a first signal, and the interaction of first label and third label (which is bound to P3) produces a second signal; and detecting the presence of at least one of the first and second signals. (M2) is useful for detecting the presence of two or more polynucleotide analytes which are alleles that differ from each other by one or more nucleotides or the insertion or deletion of one or more nucleotides. The method involves combining the polynucleotide analytes

and OP1-OP3, where OP1 comprises a sequence complementary to first region of each of the NAs, OP2 comprises a sequence complementary to second region of one of NA, and one or more OP3 comprises a sequence complementary to second region except for a difference of at least one nucleotide, or the insertion or deletion of at least one nucleotide, the difference corresponding to the one or more alleles; subjecting the reaction medium to form non-dissociative TC of at least one of (i) NA, OP1 and OP2, or (ii) NA, OP1 and the one or more OP3; and determining the presence of at least one of the TC (all claimed).

ADVANTAGE - The method can be used for the direct detection of nucleic acid in quantities as little as 10-12 or less without amplification. In addition, the method may be carried out with amplification of the target and reference sequences.

EXAMPLE - Standard PCR based protocols were used to convert 70 base single stranded synthetic wild-type (WT) and mutant (Mu) oligonucleotides into 130 base pair (bp) double-stranded amplicons. The following sequences derived from the Staphylococcus aureus mecR1 gene (Genbank Accession No.X63598) were chemically synthesized. Wild type sequence (WT) 5'-TCATTATAAAGCACAAAACTTCCATCAAATCCTTTGAAATACGGAGCTAGTTGTTTAA TTTTTTATATG, Mutant sequence (Mu) 5'-TCATTATAAAGCACTAAACTTCCATCAAATCCTTTG AAATACGGAGCTAGTTGATTTAA TTTTTTATATG. The following Polymerase chain reaction (PCR) primers were used. GO-1 (reverse linking primer for WT and Mu oligonucleotides) 5'-ATGTTCAACAAGACAAATATGAAACAAATGTATCATATAAAAAATTAAA TCAAC, GO-2 (forward linking primer for WT oligonucleotide) 5'-CTTGCTCCCGTTCATTATAAAGCACAAAAC, GT (forward linking primer for Mu oligonucleotide) 5'-CTTGCTCCCGTTCATTATAAAGCACTAAAC, FC-1 (**forward primer**) 5'-TCTGCACATGTTCAACAAGACAAAT, FC-2 (**reverse primer**) 5'-TAGAATAAGCTTGCTCCCGTTCAT. The **double-stranded DNA** produced was further increased in size by using specific primer GO-2 for WT and primer GT for Mu using similar reaction conditions except the initial denaturation was carried out at 95degreesC. The single base difference between the original WT and Mu oligonucleotides was maintained during all the primer extension and DNA amplification steps. PCR was subsequently carried out with outer primers FC-1 and FC-2 using the same reaction conditions mentioned above except that the primers were present at 250 nM. The product was analyzed on a 4-20% non-denaturing acrylamide gel. Discrimination between the Mu and WT amplicons, having a one base difference, as prepared above were carried out in the following manner. The following probes were used for detection: FC-3 (Common Probe) 3'-(T)20ATCAACTAGCTCCGTATTTCAAAG, ED-3 (Wild type specific probe) 3'-AAGTTTTGTGCTTTAA(TACT)5, ED-4 (Mutant specific probe) 3'-AAGTTTAGTGCTTTAA(CTAT)5. Detection of 100 pM WT and Mu synthetic amplicons was carried out using a Luminescent oxygen channeling immunoassay (LOCI) assay. Reactions were carried out in 40 microl reactions containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mg/ml bovine serum albumin (BSA), 4 mM MgCl2, 0.2 mM each dATP, dCTP, dTTP, dGTP. The dNTPs were added to ensure a consistent reaction medium with the PCR amplification described above. In addition the reaction also contained the 1.0 microg of chemiluminescent particle (thixone, rubene and anthracene chemiluminescent composition and chemiluminescent particle (dopTAR)-(A)20) and 1.0 microg of each of the sensitizer particles (Pc-(AGTA)6 and Nc-(ATAG)6). The common probe FC-3 binds to both the WT and Mu amplicon and to the dopTAR chemiluminescent particle (dopTAR-(A)20) via the T20 probe tail on the probe. The WT specific probe (ED-3) binds to the WT amplicon and to the Pc sensitizer particle (Pc-(AGTA)6) via the (TACT)5 tail. The Mu specific probe binds specifically to Mu amplicon and to the Nc sensitizer particle (Nc-(ATAG)6) via the (CTAT)5 tail. The chemiluminescent signal generated by the particle pairs formed was read in relative light units (RLU) by LOCI reader. The WT signal was obtained first by illuminating the reaction tubes with 675-nm laser (to generate singlet oxygen from Pc) for 1.0 second followed by reading the chemiluminescent signal. This was followed by a 10-second delay to allow the entire signal from the chemiluminescent to decay completely. The Mu signal (RLU) was then

obtained by illuminating the tubes with a 780-nm laser (to generate singlet oxygen from Nc) for one second. The Mu chemiluminescent signal was read six times after each of one-second illumination followed by one second read. The counts from each of the six reads were totaled. The background (no target) signal was subtracted from both the WT and Mu signals. The corrected signal/background counts were plotted against the amplicons added to the reaction. The results showed that the presence of both the specific probes lead to improved discrimination of the single base T to A mismatch found between the Mu and WT amplicons. (75 pages)

L5 ANSWER 11 OF 13 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-04910 BIOTECHDS
TITLE: Analysis of polynucleotides in a sample using generic capture sequences comprises amplifying target polynucleotide, and utilizing the product to indirectly assay the sample for the polynucleotide;
DNA amplification useful for pharmacogenetics, forensics, anthropology, paternity testing, functional genomics, genetic analysis, SNP, immunoglobulin mutation, pathogen detection, drug resistance, DNA sequencing anddiagnosis
AUTHOR: LAI J H; PHILLIPS V E; WATSON A R
PATENT ASSIGNEE: QUANTUM DOT CORP
PATENT INFO: WO 2001083823 8 Nov 2001
APPLICATION INFO: WO 2000-US13979 28 Apr 2000
PRIORITY INFO: US 2000-200635 28 Apr 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-114152 [15]
AN 2002-04910 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Assaying, (M1) for an amplification product (AMP) from a first target polynucleotide, (TP), comprising providing a sample that is suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a capture sequence not present in the TP at the same position, is new.

DETAILED DESCRIPTION - A target polynucleotide, (TP), is amplified, where first primer has a tag sequence, the complement of which is formed on the opposite strand during amplification and is referred to as capture sequence (CS), and the opposite strand is referred to as an amplification product (AMP) has a label; probe conjugated to substrate specific to CS, is contacted with AMP to form AMP detection complex. Assaying (M1) for an AMP from a first TP, comprises providing a sample that is suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a capture sequence not present in the TP at the same position, where the AMP is formed by primer extension from a template, where the template comprises a complement to the TP and a target noncomplementary region, where the capture sequence is a complement to the target noncomplementary region, providing a substrate that is conjugated to a first capture probe, contacting the sample with the capture probe under a first set of hybridization conditions, where the capture probe can bind to the capture sequence under the first set of hybridization conditions and determining if the first label is associated with the substrate. INDEPENDENT CLAIMS are also included for the following: (1) forming (M2) an AMP detection complex for assaying a sample for a first TP; (2) an amplification product detection complex comprising a capture probe polynucleotide hybridized to capture sequence of labeled amplification product from a TP, where the capture probe polynucleotide is conjugated to a substrate, where the capture sequence is not present in a region of the TP which is amplified and is introduced into the amplification product by copying a template polynucleotide, the template polynucleotide comprising a target noncomplementary region and a region complementary to the TP, where the target noncomplementary region was introduced into the template polynucleotide by extension of a primer hybridized to the target polynucleotide, the primer comprising the target noncomplementary region

and where the capture sequence is complementary to the target noncomplementary region; and (3) a kit for assaying for an AMP from a TP comprises a substrate attached to a capture probe, a first primer comprising a 3' end a first target complementary region located at the 3' end of the first primer, and a first target noncomplementary region that is not complementary to the first TP at a position 3' of a sequence to which the first target complementary region can hybridize, a second primer, label, housing for retaining the substrate, first primer, second primer, and a label and instruction provided with the housing that describe how to use the components of the kit to assay a sample by forming an AMP from the TP using the first and second primers that comprises the label and a capture sequence that is complementary to the target noncomplementary in the first primer, where the capture sequence can bind to the capture probe.

BIOTECHNOLOGY - Preferred Method: In (M1) the capture probe is a polynucleotide, substrate is from micorsphere, chip, slide, multiwell plate, a membrane, an optical fiber, and an optionally porous gel matrix, more preferably slide. The substrate is preferably conjugated to several different capture probe polynucleotides having corresponding different sequences, where each of the different capture probes can selectively bind to a corresponding different capture sequence on a corresponding different AMP. The substrate is preferably a first microsphere comprising a first spectral code comprising a first semiconductor nanocrystal and first fluorescence characteristics, where the first semiconductor nanocrystal comprises: (a) a core selected from ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AIAs, AIP, AIS, Ge, Si, Pb, PObSe, PbSe, their alloy or their mixture, more preferably the core is a CdSe; (b) a shell, preferably ZnS. The AMP is produced by a process comprising incorporation of a nucleotide comprising the first label into AMP using a polymerase or extension of a primer using a polymerase to form AMP, where primer comprises the first label, where the first label comprises an agent selected from chromophore; lumiphore; fluorophore preferably semiconductor nanocrystal, fluorescent dye, lanthanide chelate, a green fluorescent protein, more preferably is a lanthanide chelate selected from europium chelate, terbium chelate and a samarium chelate; chromogen; hapten; antigen; radioactive isotope; magnetic particle; metal nanoparticle; enzyme preferably alkaline phosphatase, horseradish peroxidase, beta-galactosidase, glucose oxidase, bacterial luciferase, an insect luciferase and sea pansy luciferase; antibody or binding portion or their equivalent; aptamer; and one member of a binding pair; or an agent selected from avidin, streptavidin, digoxigenin and biotin. The method preferably comprises determining if the first label, preferably a fluorophore, is associated with the substrate comprises applying a light source to the substrate that can excite the fluorophore, and determining if a fluorescence emission from the fluorophore occurs from the substrate. The sample is preferably assayed for the presence of AMP or to determine its amount, where AMP is produced at a detectably higher level from at least one allele of a locus having at least two alleles. When the substrate is a first microsphere, (M1) further comprises assaying the sample for containing a second, third and fourth AMP from a second, third and fourth TP, where second AMP from second TP and is further contacted under a second set of hybridization conditions with a second capture probe conjugated to a second microsphere, where the second capture probe is a polynucleotide, the second microsphere can be the first microsphere or a different second microsphere, the second microsphere is a different second microsphere comprising a second spectral code comprising second fluorescent characteristics, the second spectral code distinguishable from the first spectral code, the second capture probe can hybridize to the second AMP under the second set of hybridization conditions, the second AMP comprises a second label, which can be the first label when the second microsphere is a different second microsphere or can be a different second label, and determining if the second label is associated with the

second microsphere, where the first and the second AMPs are produced from a single locus, or differ by a single nucleotide. The substrate is further conjugated to a second/third/fourth capture probe, where the second/third/fourth capture probe can preferentially bind to a second/third/fourth sequence on a second/third/fourth AMP, second/third/fourth comprising a second/third/fourth second/third/fourth label that can be the same as or different than the first label and/or the second label where the binding the second/third/fourth AMP to the second/third/fourth capture probe can be independently determined. In (M2), after altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, the sample is further contacted with the first and second primers under conditions in which the first and second primers can hybridize to the second and first primer extension products, respectively, and be extended to form several first and second primer extension products, where altering the sample conditions to allow dissociation of the first and second primer extension product from the first target polynucleotide and first primer extension product, respectively comprises heating the sample. (M2) further comprising removing single-stranded polynucleotides from the sample prior to altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, where removing single-stranded polynucleotides are removed preferably by adding a thermolabile single-stranded nuclease to the sample under conditions and for a time sufficient to digest single-stranded polynucleotides in the sample, and then heating the sample to inactivate the single-stranded nuclease. In (M2), the first TP is at least one allele of a locus comprising a second allele, and where the first primer preferentially hybridizes to and is extended from the at least one allele as compared to second allele, where first and second primers has at least one mismatch with the second allele at one of the five 3' nucleotide of the first primer, and at least one allele and the second allele differ by a single nucleotide polymorphism. The method further comprises contacting the sample with a flanking primer has a lower melting point for hybridization to the first target polynucleotide than the first primer that is complementary to the first target polynucleotide and can be extended to form a flanking primer extension product, where the flanking primer is complementary to the first target polynucleotide at a flanking position that is 5' to a position at which the first target complementary region is complementary to the first target polynucleotide. (M2) further comprises forming a second AMP detection complex for assaying the sample for second TP. Utilizing a third and a fourth primer. Preferred Kit: The label is provided conjugated to a nucleotide which can be incorporated into the AMP, The label is provided conjugated to the second primer. the substrate is attached to a plurality of different capture probes, where each of said different capture probes is attached at an identifiable location on the substrate, where each of said different capture probes can preferentially hybridize to a corresponding different AMP, each of the corresponding different AMPs comprising a label that can be the same or different as the label on the other corresponding different AMPs and where the instructions further describe how to use the components to the kit to assay the sample for each of the corresponding different AMPs. M2 comprises: (a) providing a first and second primer; where first primer comprising a 3' end a first complementary region that is complementary to the first polynucleotide, first complementary region located at the 3'-end of the first primer, and a first target non complementary region that is not complementary to the first TP at a position at 3' of a sequence to which the first target complementary region can hybridize, the first primer comprising a 3'-end and a first label; (b) providing the sample the sample suspected of containing the first TP; (c) contacting the sample with the first primer under conditions in which the first target complementary region can hybridize to the first TP and the first primer can be extended to form a first primer extension product; (d) altering the sample condition to allow disassociation of the first primer

extension product from the first target polynucleotide, where the 3'-end of the first primer is complementary to the first primer extension product at a position in the first extension product that is 3' to the first target complementary region; (e) contacting the sample with the first primer under conditions in which the first can hybridize to the first primer extension product and be extended to form a first extension product comprising a first capture sequence that is the complement of the first target non complementary region and does not exist elsewhere in the first primer extension product, where the first primer extension product is the AMP; (f) altering the sample condition to allow dissociation of the first primer extension product from the first extension product; and (g) contacting the sample with a first capture probe conjugated to a first substrate where the contacting takes place under conditions in which the first capture probe can bind to the first capture sequence of the second primer extension product to form an AMP detection complex.

USE - (M2) is useful for forming an AMP detection complex for assaying a sample for first TP. The method further comprises determining if the first label is associated with the first substrate, where AMP is produced at a detectably higher level from at least one allele of a locus having at least two alleles and the first substrate preferably a first microsphere comprising a first spectral code is identified by decoding the spectral code which is preferably performed prior to, simultaneously or subsequent to determining if the first label from the second primer is associated with a substrate, first TP, preferably single-stranded or **double-stranded DNA** or RNA and a polymerase,
preferably DNA polymerase having reverse transcriptase activity is used to form the first primer extension product (claimed). (M1) is useful for particular polynucleotide sequences, whether based on SNPs, conserved sequences, or other features or useful in a wide variety of different applications. The method is useful for pharmacogenetic testing, such methods ca be used in a forensic setting to identify the species or individual which was the source of a forensic specimen. Polynucleotide analysis methods can also be used in an anthropological setting. Paternity testing is another area, as is testing for compatibility, between prospective tissue or blood donors and patients in need, and in screening for heredity disorders. (M1) is also useful for studying gene expression in response to a stimulus. Other applications include human population genetics, analyses of human evolutionary history, and characterization of human haplotype diversity. The method is useful to detect immunoglobulin class switching and hypervariable mutation of immunoglobulins, to detect polynucleotide sequences from contaminants or pathogens including bacteria, yeast, viruses, for HIV subtyping to determine the particular strains or relative amounts of particular strains infecting an individual, and can be repeatedly to monitor changes in the individuals predominant HIV strains, such as the development of drug resistance or T cell tropism; and to detect single nucleotide polymorphisms, which may be associated with particular alleles or subsets of allele. Over 1.4 million different single nucleotide polymorphisms (SNPs) in the human population have been identified. The method is also useful for mini-sequencing and for detection of mutations. Any type of mutation can be detected, including without limitation SNPs, insertions, deletions, transitions, transversions, inversions, frame shifts, triplet repeat expansions, and chromosome rearrangements. The method is useful to detect nucleotide sequences associated with increased risk of diseases or disorders, including cystic fibrosis, Tay-Sachs, sickle-cell anemia, etc. The method is useful for any assay in which a sample can be interrogated regarding an amplification product from a target polynucleotide. Typical assays involve determine the presence of the amplification product in the sample or its relative amount, or the assays may quantitative or semi-quantitative. Results from such assays can be used to determine the presence or amount of the target polynucleotide present in the sample. The above methods are particularly useful in multiplex settings where several TP are to be assayed.

EXAMPLE - 80 mul of 10 micron polysciences beads were spun down per

conjugation reaction. The supernatant was removed and the beads were resuspended in 100 mul pf imidazole buffer. The beads were spun down again and the supernatant removed. The beads were again washed in imidazole buffer. After the second wash, the beads were resuspended in 20 mul imidazole buffer. 2.0 mul of 100 muM oligonucleotide and 100 mul of 200 mM 1-(3-(dimethyl-amino)-propyl)-ethylcarbodiimide hydrochloride (EDC) made fresh imidazole buffer pH 7.0 were added to the beads. The beads were then incubated for 4 hours at room temperature with shaking. The beads were then spun down, the supernatant removed, and the beads were resuspended. This step was then repeated. The beads were then again spun down, the supernatant was removed and the beads were resuspended in 100 mul of water, this step was also repeated. The beads were then spun down, the supernatant removed and the beads were resuspended in 45 mul of PBS pH 7.4. An amplification reaction was carried out using IX Amplitaq Stoffel buffer (10 mM Tris-HCl, 10 mM KCl, pH 8.3, 200 muM each dNTP), 25 mM Magnesium chloride, 2.5 U Amplitaq Stoffel enzyme, 200 mM **reverse primer** (5'-biotin-TTCAGTGCCAACCGCCTCAC-3'), 100 nM **forward primer** (5'-GCAATAGGTTTTGAGGGGCATggttgtggaa gaggac-3'), 100 nm **forward primer** (5'-TTCTGGGCCACTGACTGATTTggttgtggaagagaac-3') (where target noncomplementary regions are in capital letters and target-specific region of the primer are in lowercase letters), 1 ng/mul genomic DNA. The heat denatured amplicon was added to 2.5 mul of conjugated beads in 85 mul of hybridization buffer. The beads was spun down, by supernatant removed and then resuspended in 100 mul 1.5XSSPE (225 mM NaCl), 0.1% sodium dodecyl sulfate. The bead were again spun down and the supernatant removed to wash away excess amplicon. The beads were then resuspended in 50 mul of PBS/BSA. 0.5 mul of 0.2 mg/ml streptavidin-Cy-Chrome dye was added and incubated with the beads for 1 hour at room temperature. The beads were then spun down and resuspended in 400 mul PBS pH 7.4. The beads were analyzed on flow cytometer in PBS pH 7.4. The unique capture sequence-tagged amplicon showed sequence-specific hybridization to its complement attached to beads. Both alleles present in the heterozygous sample could be independently amplified and identified, and heterozygous samples could be distinguished from homozygous samples. (85 pages)

L5 ANSWER 12 OF 13 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:133752 BIOSIS
DOCUMENT NUMBER: PREV200100133752
TITLE: Usefulness of PCR in situ hybridization as a technique for morphological detection of human papillomavirus in uterine cervical neoplasia.
AUTHOR(S): Oguchi, T.; Sato, S.; Xiao, Y. H.; Yokoyama, Y. [Reprint author]; Saito, Y.
CORPORATE SOURCE: Department of Obstetrics and Gynecology, Hirosaki University School of Medicine, 5-Zaifu-cho, Hirosaki, 036-8562, Japan
SOURCE: European Journal of Gynaecological Oncology, (2000) Vol. 21, No. 6, pp. 585-587. print.
ISSN: 0392-2936.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Mar 2001
Last Updated on STN: 15 Feb 2002

AB This study was designed in order to devise fitting conditions in polymerase chain reaction (PCR)-in situ hybridization (ISH) for observing human papillomavirus (HPV) infection morphologically in uterine cervical neoplasias and to compare the detection rates of HPV by PCR-ISH and solution phase PCR (S-PCR) as well as fluorescence ISH (FISH). Tissues were obtained from 23 patients with cervical intraepithelial neoplasia 3, who visited our hospital between 1994 and 1997. To detect HPV-16, a HPVpF **forward primer** and a HPVp 16 **reverse primer** were used. Compared with the traditional methods, the PCR-ISH technique performed in this study was contrived as follows. To

prevent detachment, the specimens were attached to silane-coated slides at 90degreeC and successively left at room temperature for 36 hours. In proteopepsis, pepsin was used. PCR products were fixed with 4% paraformaldehyde. PCR-ISH, S-PCR, and FISH showed HPV-16 positivity in 52.2%, 56.5% and 21.7%, respectively. The positive rate of HPV-16 detected by PCR-ISH as well as S-PCR was significantly higher than that by FISH ($p<0.01$, respectively). There was no significant difference between the positive rates of HPV-16 detected by PCR-ISH and S-PCR. HPV-16 was detected by S-PCR in all 12 specimens in which HPV-16 expression was judged as positive using PCR-ISH. Similarly, HPV-16 was found by PCR-ISH in all five specimens in which HPV-16 expression was regarded as positive using FISH. While the FISH technique detected HPV-16 signals only in the superficial and middle layers of squamous cells, the PCR-ISH technique demonstrated them in all the layers including the parabasal and basal layers. The PCR-ISH technique contrived in this study has a high sensitivity to HPV-16 equal to that of S-PCR. The difference in detection rate and distribution of HPV DNA between PCR-ISH and FISH might suggest that HPV does not infect the superficial layer but rather the parabasal layer.

L5 ANSWER 13 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:752220 CAPLUS
DOCUMENT NUMBER: 128:84884
TITLE: Direct ligation of human CD4 polymerase chain reaction fragment into vectors at specific restriction sites with positional heterostagger cloning
AUTHOR(S): Felfoldi, Ferenc; Kupihar, Zoltan; Bottka, Sandor; Puskas, Laszlo G.
CORPORATE SOURCE: Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hung.
SOURCE: Analytical Biochemistry (1997), 253(2), 275-277
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The 500-bp human CD4 fragment was cloned into XhoI and HindIII sites of pBluescript II SK after generation of the **dsDNA** fragment with sticky end overhangs on each strand. The CD4 fragment was generated by 2 sep. PCR reactions using identical forward and reverse primers; except that in one PCR, the the **forward primer** contained a 5'-terminal AGCT and in the other PCR the **reverse primer** contained a 5'-terminal TCGA. The two products were mixed, denatured, and annealed to produce 25% **dsDNA** with 5'-terminal AGCT on one strand and 5'-terminal TCGA on the complementary strand. This fragment could then be ligated into the vector previously digested with XhoI and HindIII.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 14:24:02 ON 04 JUN 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 14:24:57 ON 04 JUN 2004
L1 88 S HOFMAN S?/AU
L2 205543 S DOUBLE STRANDED DNA OR DSDNA
L3 13 S L2 AND (FORWARD PRIMER AND REVERSE PRIMER)
L4 2 S L3 AND CAPTUR?
L5 13 DUP REM L3 (0 DUPLICATES REMOVED)

=> s DNA and (forward primer and reverse primer)
L6 735 DNA AND (FORWARD PRIMER AND REVERSE PRIMER)

```
=> s nucleic acid and (forward primer and reverse primer)
L7          394 NUCLEIC ACID AND (FORWARD PRIMER AND REVERSE PRIMER)

=>

=>

=>

=>

=> s (l6 or l7) and captur###
L8           21 (L6 OR L7) AND CAPTUR###

=> s l8 and (maleamide or avidin or streptavidin or cellulose)
L9           11 L8 AND (MALEAMIDE OR AVIDIN OR STREPTAVIDIN OR CELLULOSE)

=> s l9 and (iodine-151 or radioistope or fluorophor## or fluorescen? or peptide
antigen# or antibod?)
L10           5 L9 AND (IODINE-151 OR RADIOISTOPE OR FLUOROPHOR## OR FLUORESCEN
                ? OR PEPTIDE ANTIGEN# OR ANTIBOD?)

=> dup rem l9
PROCESSING COMPLETED FOR L9
L11           9 DUP REM L9 (2 DUPLICATES REMOVED)

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12           9 DUP REM L11 (0 DUPLICATES REMOVED)

=> dup rem l10
PROCESSING COMPLETED FOR L10
L13           5 DUP REM L10 (0 DUPLICATES REMOVED)

=> d ibib abs l13 1-5

L13  ANSWER 1 OF 5  CAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2004:162310  CAPLUS
DOCUMENT NUMBER:          140:176229
TITLE:                    Diagnostic polymerase chain reaction process utilizing
                          simultaneous capture and detection of
                          amplicons
INVENTOR(S):              Hofmann, Scott Daniel
PATENT ASSIGNEE(S):       USA
SOURCE:                   U.S. Pat. Appl. Publ., 4 pp.
                          CODEN: USXXCO
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.   DATE
     ---------------   ----  --------       ---------------   --------
     US 2004038194      A1   20040226       US 2001-974648    20011009
PRIORITY APPLN. INFO.:                   US 2000-238792P  P  20001006
AB   A method simultaneously detects and captures double-stranded
     DNA (dsDNA) sequence.  The method includes adding a
     forward primer and a reverse primer
     for the dsDNA sequence in a sample; either the forward
     primer or the reverse primer have a
     capture agent, and the other has a detection agent.  Replication
     of the dsDNA by PCR results in a segment of dsDNA with one specific end
     designed to be captured onto a medium and the opposite end
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designed for detection. The method is intrinsic and simultaneous and lends itself to ease of purification after **capture** and prior to detection and subsequent quantification. The method also can be applied to dsDNA that has been reverse -transcribed from single-stranded RNA. This procedure is exemplified by detecting an HIV viral load.

L13 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-13777 BIOTECHDS
TITLE: Determining the genetic haplotype of a part of an individual genotype, comprises determining the presence or absence of specific nucleotide polymorphisms of a pair of homologous chromosomes;
with use of polymerase chain reaction and ligase chain reaction
AUTHOR: FENGER M; BENTZEN J
PATENT ASSIGNEE: HVIDOVRE HOSPITAL
PATENT INFO: WO 2003018835 6 Mar 2003
APPLICATION INFO: WO 2002-DK552 22 Aug 2002
PRIORITY INFO: DK 2001-1252 23 Aug 2001; DK 2001-1252 23 Aug 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-342453 [32]
AN 2003-13777 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Detecting the presence/absence of multiple haplotypic genetic variations in a preselected region of one chromosome of a chromosome pair comprises selecting nucleotide polymorphisms, suspected to occur in the preselected region, isolating a target **nucleic acid** (TNA) of the chromosome pair from a sample, and detecting presence/absence of several polymorphisms, using designed oligonucleotide probes.

DETAILED DESCRIPTION - Detecting the presence/absence of multiple haplotypic genetic variations in a preselected region of one chromosome of a chromosome pair comprises: (a) selecting several nucleotide polymorphisms suspended to occur in the preselected region; (b) designing a set of oligonucleotide probe members, each of which is capable of hybridizing specifically with one individually selected nucleotide sequence in the preselected region comprising at least one polymorphism suspected to occur in the preselected region; (c) obtaining from an individual a **nucleic acid** sample which contains a target **nucleic acid** comprising the preselected region of the one member or a chromosome pair; (d) isolating or specifically analyzing at least part of the target **nucleic acid** of the one member of a chromosome pair comprising the preselected region, thus obtaining a resolution of the haplotypes from the individual; and (e) detecting the presence or absence of several polymorphisms in the preselected region by **nucleic acid** hybridization with the set of oligonucleotide probe members. An INDEPENDENT CLAIM is also included for a kit for detecting the presence or absence of multiple haplotypic genetic variation in a preselected region of one chromosome of a chromosome pair.

BIOTECHNOLOGY - Preferred Method: One probe of the set is an allele-specific **capture** probe or primer, which is specific for one allele of a first polymorphism in the preselected region, and the other probe of the set is an allele specific detection-probe which is specific for one allele of a second polymorphism in the preselected region. The method further comprises isolating at least a part of the target **nucleic acid** sequence by **capturing** the target **nucleic acid** sequence with the allele specific **capture** probe, or isolating the target **nucleic acid** comprises amplifying it using a primer pair of which one member is the allele specific primer, and obtaining a resolution of the haplotypes, where the probe is differently labeled. The method further comprises detecting the presence or absence of several polymorphisms by

contacting the amplified target **nucleic acid** with the allele specific detection probe, and detecting the hybrid between the target **nucleic acid** and the allele specific detection probe. Further, the method comprises providing **capture** probes specific for every allele of a first polymorphism, where the **capture** probes are immobilized in different positions to enable the resolution of both haplotypes of a diploid individual, or the amplification of every allele of a first polymorphism to separate amplification reactions to enable resolution of both haplotypes of a diploid individual. The method comprises specifically analyzing the target **nucleic acid** by contacting the target with the multifunctional detection probe, and obtaining a resolution of the haplotypes, where the multifunctional detection probes can detect every combination of alleles for the multiple polymorphisms. The target **nucleic acid** is selected from genomic **DNA**, cDNA, mRNA, mitochondrial **DNA** and chloroplast **DNA**. At least one member of the set of oligonucleotide probes are labeled with a **fluorescent** reporter group (fluorescein, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, acridin, Hoechst 33258, rhodamine, rhodamine green, tetramethylrhodamine, Texas red, cascade blue, Oregon green, Alexa flour, europium, and samarium), with an enzyme tag (beta-galactosidase, peroxidase, horseradish peroxidase, urease, glycosidase, alkaline phosphatase, chloramphenicol acetyltransferase and luciferase), with a chemiluminescent group (luminol and oxalate esters), or with a radioisotope (hydrazides and the radioisotope is selected from 32P, 32P, 35S, 125I, 45Ca, 14C and 3H). Isolation of the resolved haplotype comprises the binding of at least one member of the set of oligonucleotide probes to a solid surface, where the preselected region is amplified, preferably by an allele specific amplification method. The amplification is performed by PCR, Ligase Chain Reaction (LCR), **Nucleic Acid** Sequence-Based Amplification (NASBA), strand displacement amplification, rolling circle amplification, and T7-polymerase amplification. The method further comprises the use of an allele-specific primer coupled to an entity suitable for a subsequent immobilization reaction, performing the amplification reaction, and thus obtaining an amplified **DNA** molecule with the entity coupled to one of the **DNA** strands, dissociating the two strands of the amplified **DNA** molecule, binding only the coupled **DNA** strand to a solid surface, and contacting the bound **DNA** with several differently labeled oligonucleotide probes each of which is capable of detecting one specific polymorphism by **nucleic acid** hybridization. The solid surface is selected from nitrocellulose surface, a **cellulose** surface, a diazotized surface and a nylon surface. Binding further comprises coupling one member of an affinity pair to the **nucleic acid** while the other member of the affinity pair is immobilized on the solid surface. The affinity pair is selected from biotin-**streptavidin**, biotin-**avidin**, digoxigenin-anti-hapten **antibody**, fluorescein-anti-hapten **antibody**, lectins-lectin receptor, ion-ion chelators, immunoglobulin (Ig) G-protein A, IgG-protein G and magnets-paramagnetic particles. Binding comprises the coupling of the **nucleic acid** to a photoreactive group, or chemoreactive group. The photoreactive group is selected from quinone, an anthraquinone, psoralene, coumarin, benzofuran, indole and a photoactivable ketone, including benzophenone and acetophenone. The chemoreactive group is selected from sulfhydryl, a primary amine and a primary phosphate. The resolution of haplotypes of a preselected region of the one member of a chromosome pair is performed by hybridization techniques in a one-phase system. Several nucleotide polymorphisms of the preselected region is detected by hybridization in suspension with one labeled oligonucleotide probe which is capable of detecting several polymorphisms, such as at least 2 polymorphisms, for e.g., up to 10 polymorphisms. Several nucleotide polymorphisms are detected by hybridization in suspension with several different labeled

oligonucleotide probes each of which is capable of detecting one specific polymorphism. The preselected region is amplified by the above mentioned allele specific amplification methods. In the method, the spacer comprises 8-28 nucleotides, where two adjacent polymorphisms are separated by at least 15- 150 nucleotides, preferably at least 200 nucleotides, for e.g., at least 250 nucleotides, or more preferably up to 1000 kb. Preferred Kit: The kit comprises: (a) at least two oligonucleotide probes, each of which is capable of hybridizing specifically with one allele of an individually selected polymorphic locus occurring in the preselected region, one of the probe is a detection probe, which is bound to a label, two adjacent polymorphic loci is separated by at least 15 nucleotides; and (b) buffers and solutions for carrying out hybridization under conditions of high stringency between the oligonucleotide probes and a target **nucleic acid** sequence comprising the preselected region. One probe is a **capture** probe coupled to an entity suitable for a subsequent immobilization reaction and the other probe is a detection probe. The kit comprises a primer pair, one of which is an allele specific primer, which is an oligonucleotide probe, and an allele specific probes to resolve all possible combinations of allele for the two or more polymorphisms. The kit further comprises multifunctional oligonucleotide probe comprising: (a) a first allele specific nucleotide sequence, which is specific for one allele of a first polymorphic locus; (b) a second allele specific nucleotide sequence, which is specific for one allele of a second polymorphic locus; (c) a spacer sequence, which does not hybridize to any part of the target **nucleic acid** under conditions where the allele specific sequences hybridize, and which separates the first and second allele specific nucleotide sequences; and (d) a detectable label.

USE - The method is useful for detecting the presence or absence of multiple haplotypic genetic variations in a preselected region of one chromosome of a chromosome pair (claimed). The method is also useful for determining the linkage phase between two adjacent loci.

ADVANTAGE - The method is fast, inexpensive and provides uncomplicated determination of haplotypes in **nucleic acid** fragments of varying length from 25-40000 base pairs.

EXAMPLE - Detection of ApoB haplotype by hybridization to an immobilized detection probe was as follows. Human genomic **DNA** was isolated from 5 ml mammalian full blood, two primers, **forward primer**: 5'-GATGAAACCAATGACAAAATCC-3', and **reverse primer**: 5'-TGCTCCGTTCTCAGGTACTTGC-3' spanning the region of the 2488 and 2712 polymorphisms were designed and PCR amplification was carried out. Two oligonucleotides, 21 base pairs long, biotinylated in the 5' and which were complementary to either the C-allele or the T-allele of the rodon 2488 polymorphisms were designed. These oligos were attached to a microtiter plate well coated with **streptavidin**. The two oligos were placed on separate surfaces (e.g. the one complementary to the C-allele in one microtiter plate well and the one complementary to the T-allele in a different well). The PCR amplicon was added to the oligonucleotides attached to the solid surface and allowed to hybridize. The surfaces were shaken and then washed with 200 microl 1xSSCT buffer 5 times. The PCR-amplicons, which do not contain a perfect match for the attached oligonucleotide would not have hybridized and was therefore washed off, while the PCR-amplicons, which contained a sequence 100% complementary to the one possessed by the oligonucleotide was hybridized. (56 pages)

L13 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-00777 BIOTECHDS
TITLE: New isolated melanocortin-5 receptor useful for identifying a compound that binds to melanocortin-5 receptor and identifying antagonists/agonists of canine melanocortin-5 receptor useful for treating skin disorders; recombinant protein production and antagonist and agonist

for use in disease therapy and gene therapy

AUTHOR: HOUSEKNECHT K L; ROBERTSON A S; XIAO Y
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AN 2004-00777 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated melanocortin-5 receptor (MC5R) polypeptide comprising an amino acid sequence chosen from a fully defined sequence of 325 amino acids, as given in the specification, an amino acid sequence encoded by canine MC5R clone, and an extracellular or cytoplasmic domain of canine MC5R or of polypeptide encoded by canine MC5R clone, is new.

DETAILED DESCRIPTION - An isolated canine melanocortin-5 receptor (MC5R) polypeptide (I) comprising an amino acid sequence chosen from a fully defined sequence of 325 amino acids (S1) as given in the specification, an amino acid sequence encoded by canine MC5R clone, an extracellular domain of a canine MC5R corresponding to amino acids 1-37, 90-119, 181-185, or 265-272 of (S1) or of polypeptide encoded by canine MC5R clone, and a cytoplasmic domain of a canine MC5R corresponding to amino acids 63-70, 139-160, 213-237, or 297-325 of (S1) or of polypeptide encoded by canine MC5R clone deposited with the ATCC having ATCC Accession Number PTA-3455 (A1). INDEPENDENT CLAIMS are also included for: (1) an isolated **nucleic acid** (II) encoding a functional MC5R, or its complement, the **nucleic acid** comprising a nucleotide sequence chosen from a nucleotide sequence which hybridizes under conditions of moderate stringency to the coding region of a fully defined sequence of 3945 nucleotides (S2) as given in the specification, a nucleotide sequence which hybridizes under conditions of moderate stringency to a polynucleotide which is complementary to the coding region of (S2), a nucleotide sequence which hybridizes under conditions of moderate stringency to the coding region of the canine MC5R as deposited with (A1), and a nucleotide sequence which hybridizes under conditions of moderate stringency to a polynucleotide which is complementary to the coding region of the canine MC5R as deposited with (A1), provided that the functional MC5R is not human, bovine, chimpanzee, murine, rat, chicken or swine; (2) an isolated **nucleic acid** encoding a functional MC5R, or its complement, the **nucleic acid** comprising a nucleotide sequence chosen from a nucleotide sequence which hybridizes under conditions of high stringency to (S2), a nucleotide sequence which hybridizes under conditions of high stringency to a polynucleotide which is complementary to the coding region of (S2), a nucleotide sequence which hybridizes under conditions of high stringency to the coding region of the canine MC5R as deposited with (A1), and a nucleotide sequence which hybridizes under conditions of high stringency to a polynucleotide which is complementary to the coding region of the canine MC5R as deposited with (A1); (3) an isolated **nucleic acid** (III) comprising a nucleotide sequence that encodes a polypeptide having (S1), or encodes a polypeptide encoded by the canine MC5R clone as deposited with (A1); (4) an isolated **nucleic acid** comprising a nucleotide sequence having more than 87 % identity to (S1) or a fully defined sequence of 978 nucleotides (S3) as given in the specification or to the canine MC5R clone as deposited with (A1); (5) an isolated **nucleic acid** comprising a nucleotide sequence encoding an extracellular domain of a canine MC5R corresponding to amino acids 1-37, 90-119, 181-185, or 265-272 of (S1) or of the polypeptide encoded by the canine MC5R clone as deposited with (A1); (6) an isolated **nucleic acid** comprising a nucleotide sequence encoding a cytoplasmic domain of a canine MC5R corresponding to amino acids 63-70, 139-160, 213-217, or 297-325 of (S1) or of the polypeptide encoded by the canine

MC5R clone as deposited with (A1); (7) a nucleotide vector (IV) comprising (II) under the control of a nucleotide regulatory element with which the **nucleic acid** is not naturally associated;
(8) a genetically engineered host cell (V) that expresses functional MC5R and comprises (II); (9) an isolated polypeptide (VI) encoded by (II); (10) identifying a compound that binds to (VI); (11) an **antibody** (VII) that immuno-specifically binds a polypeptide having (S1); (12) producing (I); (13) detecting a polynucleotide comprising (II); (14) identifying (ant)agonists of canine MC5R; (15) preparing recombinant canine MC5R polypeptide; (16) a composition (VIII) comprising a substantially pure polypeptide having (S1); (17) a transgenic animal, the nucleated cells of which comprise a transgene encoding (VI); and (18) a composition (IX) comprising an agonist or antagonist of canine MC5R identified by (I) and a carrier.

BIOTECHNOLOGY - Preparation (claimed): (I) is prepared by culturing a host cell transformed with an expression vector which expresses the recombinant canine MC5R polypeptide in the host cell membranes, isolating membranes having (I) from the host cell and recovering the recombinant canine MC5R polypeptide from the cell culture. Preferred **Nucleic Acid**: In (III), the **nucleic acid** has (S2), (S3) or the canine MC5R clone as deposited with (A1). Preferred Host Cell: In (V), the **nucleic acid** is in operative association with a nucleotide regulatory element that controls expression of the nucleotide sequence in the host cell. Preferred Method: Detecting a polynucleotide comprising (II) in a sample comprises contacting the sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, the polynucleotide is detected. (II) is useful for detecting a polynucleotide comprising (II) in a sample comprising contacting the sample under stringent hybridization conditions with **nucleic acid** primers that anneal to the **nucleic acid** under such conditions, and amplifying the annealed polynucleotides, so that if a polynucleotide is amplified, the polynucleotide is detected. The polynucleotide is an RNA molecule that encodes a functional MC5R, and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide. Identifying a compound that binds to (VI) comprises contacting a compound with (VI) for a time sufficient to form a polypeptide/compound complex, and determining whether the polypeptide/compound complex is formed, where if the polypeptide/compound complex is formed, then a compound that binds to the polypeptide is identified. Alternatively, the method comprises contacting a compound with the polypeptide in a cell, for a time sufficient to form a polypeptide/compound complex, where the complex drives expression of a reporter gene sequence in the cell, and detecting the complex by detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds to the polypeptide is identified. Identifying antagonists of canine MC5R comprises contacting a cell line that expresses (VI) with a test compound in the presence of an MC5R agonist, and determining whether the test compound inhibits binding of the MC5R agonist to the MC5R or inhibits functional activity of the MC5R, in which antagonists are identified as those compounds that inhibit binding of the MC5R agonist to the MC5R or inhibit functional activity of the MC5R. Identifying agonists of canine MC5R comprises contacting a cell line that expresses (VI) with a test compound in the presence and in the absence of an MC5R agonist, determining whether, in the presence of the MC5R agonist, the test compound inhibits the binding of the MC5R agonist to the cell line, and determining whether, in the absence of the MC5R agonist, the test compound mimics the cellular effects of the MC5R agonist on the cell line, in which agonists are identified as those test compounds that inhibit the binding but mimic the cellular effects of the MC5R agonist on the cell line.

ACTIVITY - Anabolic; Eating-Disorders-Gen.; Immunomodulator;

Dermatological; Antiinflammatory; Antidiabetic; Anorectic; Antipruritic. No biological data given.

MECHANISM OF ACTION - Modulating appetite and/or metabolic rate. No biological data given.

USE - An isolated **nucleic acid** (II) encoding a functional MC5R is useful for detecting a polynucleotide comprising (II) in a sample. An isolated polypeptide (VI) encoded by (II) is useful for identifying a compound that binds to (VI). (VI) is useful for identifying (ant)agonists of canine MC5R. A composition (IX) comprising an agonist or antagonist of canine MC5R(IX) is useful for modulating the appetite and/or metabolic rate, or treating skin disorders of an animal in need of it which involves administering to the animal an MC5R ligand. The animal suffers from a decreased-appetite disorder and the MC5R ligand administered is an MC5R antagonist. The disorder is anorexia or cachexia. The animal suffers from type 2 diabetes or obesity and the MC5R ligand administered is an MC5R agonist. The skin disorder is seborrhea and the MC5R ligand administered is an antagonist. The skin disorder is pruritis or allergic dermatitis and the MC5R ligand administered is an MC5R agonist (all claimed).

ADMINISTRATION - A composition (IX) is administered by inhalation, oral, buccal, parenteral, topical, transdermal or rectal routes. No dosage given.

EXAMPLE - Canine skin tissue were prepared. cDNA libraries were prepared by Life Technologies. A biotinylated **capture** oligonucleotide had the sequence CATAGGGGCCATAGTGAAGAACAAAAACC which was designed from a conserved region of the isolated melanocortin-5 receptor (MC5R) polynucleotide sequence. Canine MC5R clones were isolated from their corresponding cDNA libraries using the oligonucleotide and the GENETRAPPER (RTM) system. Positive selection was achieved by stringently hybridizing the oligonucleotide to clones in a cDNA library. The complex was separated from all other clones in the library using **Streptavidin** magnetic beads, which were pelleted using a magnet. MC5R clones were identified by PCR using a MC5R specific primer pair designed to be outside of the **capture** oligonucleotide site (**forward primer**: TTCGCCGTGTGAAGAGATGG; **reverse primer**: ACGCCTCACGGTCATGATGT). One canine MC5R clone was isolated from the cDNA libraries, yielding a clone of 3.9 kilobases in size. Identity of the clones was confirmed by PCR using a different MC5R polynucleotide sequence-specific primer pair (**forward primer**: TGAGGCGTTCCAGAGTGTTAT, **reverse primer** : GGAGCCCAGCACACGAT). Sequencing of the canine MC5R polynucleotide was completed using one standard and seven primer walking reactions from both ends of each clone. Sequences of the MC5R polynucleotide fragments were assembled based on their overlapping regions. The nucleotide sequence encoding canine MC5R was depicted as a fully defined sequence of 3945 nucleotides as given in the specification. Conceptual translation of the open reading frame (ORF) of the nucleotide sequence revealed the predicted amino acid sequence of canine MC5R as a fully defined sequence of 325 amino acids as given in the specification. The open reading frame nucleotide sequence was depicted as a fully defined sequence of 978 nucleotides as given in the specification. (35 pages)

L13 ANSWER 4 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-00844 BIOTECHDS
TITLE: Detecting if an organism is homozygous or heterozygous in a target sequence, by combining differential hybridization or restriction endonuclease digestion with immobilized array technology or electrophoretic separation;
gene mutation detection in plant, mammal or human using **DNA** primer and **DNA** probe for disease therapy and propagation
AUTHOR: SIEMERING K
PATENT ASSIGNEE: MURDOCH CHILDRENS RES INST
PATENT INFO: WO 2002050305 27 Jun 2002

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AN 2003-00844 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Determining (M) presence or absence of a homozygous or heterozygous change in one or more nucleotides within a target nucleotide sequence, using forward and reverse primers which are labeled with reporter molecules which provide separate identifiable signals, or unlabeled primers where detection is accomplished by hybridization of a probe labeled at its 5' and 3' termini, is new.

DETAILED DESCRIPTION - Determining (M) presence or absence of a homozygous or heterozygous change in one or more nucleotides within a target nucleotide sequence, comprising: (a) amplifying target nucleotide sequence using forward and reverse primers to produce an amplified product, where at least one of the primers is labeled with a reporter molecule capable of facilitating the provision of an identifiable signal which can be distinguished from another receptor molecule if both primers are labeled, and at least one primer and its complementary form comprises a complementary sequence to an oligonucleotide sequence anchored to a solid support, and the primers introduce, abolish or hybridize to a target site within the amplified product in the presence or absence of a change in one or more nucleotides, and subjecting the amplified product to detection unit; or (b) the method of: (i) amplifying the target nucleotide sequence using forward and reverse primers to produce an amplified product, where one primer comprises one or more chemically modified nucleotides, bases or phosphodiester bonds so that a nucleotide strand which extends from the primer is resistant to exonuclease activity and the other primer comprises a nucleotide sequences having sense and complementary sequences immobilized to a solid support, where the primers introduces or abolishes a restriction endonuclease site within the amplified product in the presence or absence of a change in one or more nucleotides; (ii) digesting the amplified product with an exonuclease to digest the strand not amplified by the primer comprising the exonuclease-resistant nucleotides, bases or phosphodiester linkages to generate a single-stranded **nucleic acid** molecule comprising the potential presence or absence of a restriction endonucleases site and a nucleotide sequence complementary to an oligonucleotide sequence immobilized to the solid support; (iii) hybridizing to the single stranded **nucleic acid** molecule a probe that contains complementary to the restriction site that may have been introduced to generate a partial double-stranded molecule, where the probe comprises two reporter molecules capable of facilitating the provision of identifiable signals which can be distinguished from each other; (iv) digesting the partially double-stranded molecule with the restriction endonuclease whose site has been potentially introduced or abolished in the amplified product and subjecting the digested molecule to conditions to permit annealing to a set of the immobilized oligonucleotides comprising oligonucleotides which are sense or complementary to a portion of the amplified sequence introduced by at least one primer; and (v) detecting the relative proportion of signal by the reporter molecules, where an equal proportion of different signals or the presence of only one signal represents a homozygous presence or absence of change in the target nucleotide sequence and the presence of a differential signal represents a heterozygous presence or absence of the change in target nucleotide sequence. An INDEPENDENT CLAIM is also included for an assay device for determining the presence or absence of a nucleotide or group of nucleotides in a **nucleic acid** molecule, comprising an array of immobilized oligonucleotides each complementary to a nucleotide sequence within an amplified product digested by one or more restriction endonucleases and unit to screen for the hybridization of a target sequence to the immobilized oligonucleotide

array.

BIOTECHNOLOGY - Preferred Method: In (Mi), the detection unit comprises detecting the relative proportion of signal or lack of signal by the reporter molecules. The target site within the amplified product is a restriction endonuclease site. One or more of the forward or reverse primers introduces a restriction endonuclease site within the amplified product. The solid support is glass or a polymer such as **cellulose**, nitrocellulose, ceramic material, polyacrylamide, nylon, polystyrene and its derivatives, polyvinylidene difluoride, methacrylate and its derivatives, polyvinylchloride and polypropylene, preferably glass. Two or more oligonucleotide sequences are anchored to the solid support in the form of an array. The restriction endonuclease site is recognized by restriction enzymes listed in the specification such as AatI, AatII, AauI, BaeI, BamHI, Cac8I, CbiI, DraIII, DsaI, Eco1301, EcoRI, Esp3I, FspI, GsuI, HphI, ItaI, Ksp22I, LspI, MseI, NarI, PacI, RsaI, SecI, TaqII, UbaDI, Van91I, XagI, Zsp2I, etc. The reporter molecule is chloramphenicol, colorless galactosidase, colorless glucuronide, luciferin, and green **fluorescent** protein. Alternatively, the differential restriction endonuclease digestion is assessed electrophoretically, where the pattern of electrophoretic separation and/or the pattern of reporter molecule signaling is indicative of homozygous or heterozygous presence or absence of change in the target sequence. Preferred Device: The device is packaged for sale and contains instructions for use.

USE - (M) is useful for determining the presence or absence of a homozygous or heterozygous change in one or more nucleotides within a target nucleotide sequence. The target sequence is in a eukaryotic cell such as a plant cell or a mammalian cell in particular a human cell, and the target sequence is associated with a disease condition comprising one or more known genetic mutations. The disease condition is adreno-leukodystrophy, atherosclerosis, gaucher disease, gyrate atrophy, juvenile onset diabetes, obesity, paroxysmal nocturnal hemoglobinuria, phenylketonuria, refsum disease, tangler disease and hemochromatosis conditions involving transporters, channels and pumps such as cystic fibrosis, deafness, diastrophic dysplasia, long-QT syndrome, Menkes syndrome, Pendred syndrome, polycystic kidney disease, sickle cell anemia, Wilson's disease and Zellweger syndrome, conditions involving signal transduction such as ataxia telangiectasia, baldness, Cockayne syndrome, glaucoma, tuberous sclerosis, Waardenburg syndrome and Werner syndrome, conditions involving the brain such as Alzheimer's disease, amyotrophic lateral sclerosis, Angleman syndrome, Charcot-Marie-Tooth disease, epilepsy, essential tremor, fragile X syndrome, Friedreich's ataxia, Huntington's disease, Niemann-Pick disease, Parkinson's disease, Prader-Willi syndrome, Rett syndrome, spinocerebellar atrophy and William's syndrome, and conditions involving the skeleton such as Duchenne muscular dystrophy, Ellis-van Creveld syndrome, Marfan syndrome and myotonic dystrophy. (All claimed). (M) is useful for screening for polymorphic variants in the genome of plants such as during the tissue culture stages of plant propagation. The ability to identify polymorphic variants in plants such as due to somaclonal variation prevents unnecessary resources being wasted on plants with undesired properties.

EXAMPLE - A mutation at nucleotide 35 in the connexin 26 gene was identified either in the homozygous or heterozygous state. The mutation was a deletion of a guanine at position 35. This mutation was referred to as 35DELTAG. Two primers were developed, each labeled with a different reporter molecule and at least one comprising a nucleotide sequence matching and complementary to oligonucleotide sequences immobilized to a solid support. This sequence on the primer was referred to as a tag sequence. The primers comprised a reporter molecule alone or linked to a tag sequence having matching and complementary sequences immobilized to a solid support. One primer comprised a tag sequence linked to a nucleotide sequence complementary to a region flanking the 35DELTAG region for the **forward primer** and a region downstream of this location for the **reverse primer**. The **reverse**

primer introduced a base change in the wild-type sequence thus creating a EcoRII site. If the target sequence comprised a 35 DELTAG mutation then the EcoRII site was lost, as EcoRII recognized the nucleotide sequence 5'CCWGG3', where W is A or T. In the connexin 26 gene, the nucleotide sequence recognized by EcoRII was 5'CCTGG3'. A 35 DELTAG mutation removed the G at the 3' position and hence, amplification product from a 35 DELTAG sample did not digest, while the wild-type sequence digested. After amplification and digestion with EcoRII, single-stranded forms of the amplified product were exposed to the immobilized oligonucleotides on the solid support. When the target sequence was homozygous wild-type, all the amplification product were digested thus removing the reporter molecule associated with the **reverse primer**. The complementary immobilized oligonucleotide (+) permitted **capture** of the tag associated with the **forward primer**. The matching (sense) immobilized oligonucleotide (-) permitted **capture** of the sequence complementary to the tag generated by extension of the **reverse primer** during polymerase chain reaction (PCR). As the reporter molecule associated with the **reverse primer** was cleaved away by EcoRII digestion, no reporter molecule was detected at the (-) feature of the immobilized oligonucleotide pair, i.e. the ratio of signal from forward to **reverse primer** was in the order of 1:0. When the 35 DELTAG mutation was present in a homozygous state, there was no digestion of any amplification product and both reporter molecules on both primers were equally represented, i.e. in a ratio of 1:1. When the 35DELTAG mutation was in the heterozygous state, then the amplification product from the nucleotide sequence carrying the mutation was not cleaved but cleavage occurred in the amplification product from the nucleotide sequence not carrying the mutation. About half of the molecules in the total amplification were cleaved. Accordingly, the ratio of signal from forward to **reverse primer** was 1:0.5. (73 pages)

L13 ANSWER 5 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-04910 BIOTECHDS
TITLE: Analysis of polynucleotides in a sample using generic **capture** sequences comprises amplifying target polynucleotide, and utilizing the product to indirectly assay the sample for the polynucleotide;
DNA amplification useful for pharmacogenetics, forensics, anthropology, paternity testing, functional genomics, genetic analysis, SNP, immunoglobulin mutation, pathogen detection, drug resistance, **DNA** sequencing anddiagnosis
AUTHOR: LAI J H; PHILLIPS V E; WATSON A R
PATENT ASSIGNEE: QUANTUM DOT CORP
PATENT INFO: WO 2001083823 8 Nov 2001
APPLICATION INFO: WO 2000-US13979 28 Apr 2000
PRIORITY INFO: US 2000-200635 28 Apr 2000
DOCUMENT TYPE: Patent
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OTHER SOURCE: WPI: 2002-114152 [15]
AN 2002-04910 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Assaying, (M1) for an amplification product (AMP) from a first target polynucleotide, (TP), comprising providing a sample that is suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a **capture** sequence not present in the TP at the same position, is new.
DETAILED DESCRIPTION - A target polynucleotide, (TP), is amplified, where first primer has a tag sequence, the complement of which is formed on the opposite strand during amplification and is referred to as **capture** sequence (CS), and the opposite strand is referred to as an amplification product (AMP) has a label; probe conjugated to substrate

specific to CS, is contacted with AMP to form AMP detection complex. Assaying (M1) for an AMP from a first TP, comprises providing a sample that is suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a **capture** sequence not present in the TP at the same position, where the AMP is formed by primer extension from a template, where the template comprises a complement to the TP and a target noncomplementary region, where the **capture** sequence is a complement to the target noncomplementary region, providing a substrate that is conjugated to a first **capture** probe, contacting the sample with the **capture** probe under a first set of hybridization conditions, where the **capture** probe can bind to the **capture** sequence under the first set of hybridization conditions and determining if the first label is associated with the substrate. INDEPENDENT CLAIMS are also included for the following: (1) forming (M2) an AMP detection complex for assaying a sample for a first TP; (2) an amplification product detection complex comprising a **capture** probe polynucleotide hybridized to **capture** sequence of labeled amplification product from a TP, where the **capture** probe polynucleotide is conjugated to a substrate, where the **capture** sequence is not present in a region of the TP which is amplified and is introduced into the amplification product by copying a template polynucleotide, the template polynucleotide comprising a target noncomplementary region and a region complementary to the TP, where the target noncomplementary region was introduced into the template polynucleotide by extension of a primer hybridized to the target polynucleotide, the primer comprising the target noncomplementary region and where the **capture** sequence is complementary to the target noncomplementary region; and (3) a kit for assaying for an AMP from a TP comprises a substrate attached to a **capture** probe, a first primer comprising a 3' end a first target complementary region located at the 3' end of the first primer, and a first target noncomplementary region that is not complementary to the first TP at a position 3' of a sequence to which the first target complementary region can hybridize, a second primer, label, housing for retaining the substrate, first primer, second primer, and a label and instruction provided with the housing that describe how to use the components of the kit to assay a sample by forming an AMP from the TP using the first and second primers that comprises the label and a **capture** sequence that is complementary to the target noncomplementary in the first primer, where the **capture** sequence can bind to the **capture** probe.

BIOTECHNOLOGY - Preferred Method: In (M1) the **capture** probe is a polynucleotide, substrate is from micorsphere, chip, slide, multiwell plate, a membrane, an optical fiber, and an optionally porous gel matrix, more preferably slide. The substrate is preferably conjugated to several different **capture** probe polynucleotides having corresponding different sequences, where each of the different **capture** probes can selectively bind to a corresponding different **capture** sequence on a corresponding different AMP. The substrate is preferably a first microsphere comprising a first spectral code comprising a first semiconductor nanocrystal and first **fluorescence** characteristics, where the first semiconductor nanocrystal comprises: (a) a core selected from ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AlAs, AlP, AlS, Ge, Si, Pb, PObSe, PbSe, their alloy or their mixture, more preferably the core is a CdSe; (b) a shell, preferably ZnS. The AMP is produced by a process comprising incorporation of a nucleotide comprising the first label into AMP using a polymerase or extension of a primer using a polymerase to form AMP, where primer comprises the first label, where the first label comprises an agent selected from chromophore; lumiphore; **fluorophore** preferably semiconductor nanocrystal, **fluorescent** dye, lanthanide chelate, a green **fluorescent** protein, more preferably is a lanthanide chelate selected from europium chelate, terbium chelate and a samarium chelate; chromogen; hapten;

antigen; radioactive isotope; magnetic particle; metal nanoparticle; enzyme preferably alkaline phosphatase, horseradish peroxidase, beta-galactosidase, glucose oxidase, bacterial luciferase, an insect luciferase and sea pansy luciferase; **antibody** or binding portion or their equivalent; aptamer; and one member of a binding pair; or an agent selected from **avidin**, **streptavidin**, digoxigenin and biotin. The method preferably comprises determining if the first label, preferably a **fluorophore**, is associated with the substrate comprises applying a light source to the substrate that can excite the **fluorophore**, and determining if a **fluorescence** emission from the **fluorophore** occurs from the substrate. The sample is preferably assayed for the presence of AMP or to determine its amount, where AMP is produced at a detectably higher level from at least one allele of a locus having at least two alleles. When the substrate is a first microsphere, (M1) further comprises assaying the sample for containing a second, third and fourth AMP from a second, third and fourth TP, where second AMP from second TP and is further contacted under a second set of hybridization conditions with a second **capture** probe conjugated to a second microsphere, where the second **capture** probe is a polynucleotide, the second microsphere can be the first microsphere or a different second microsphere, the second microsphere is a different second microsphere comprising a second spectral code comprising second **fluorescent** characteristics, the second spectral code distinguishable from the first spectral code, the second **capture** probe can hybridize to the second AMP under the second set of hybridization conditions, the second AMP comprises a second label, which can be the first label when the second microsphere is a different second microsphere or can be a different second label, and determining if the second label is associated with the second microsphere, where the first and the second AMPs are produced from a single locus, or differ by a single nucleotide. The substrate is further conjugated to a second/third/fourth **capture** probe, where the second/third/fourth **capture** probe can preferentially bind to a second/third/fourth sequence on a second/third/fourth AMP, second/third/fourth comprising a second/third/fourth second/third/fourth label that can be the same as or different than the first label and/or the second label where the binding the second/third/fourth AMP to the second/third/fourth **capture** probe can be independently determined. In (M2), after altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, the sample is further contacted with the first and second primers under conditions in which the first and second primers can hybridize to the second and first primer extension products, respectively, and be extended to form several first and second primer extension products, where altering the sample conditions to allow dissociation of the first and second primer extension product from the first target polynucleotide and first primer extension product, respectively comprises heating the sample. (M2) further comprising removing single-stranded polynucleotides from the sample prior to altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, where removing single-stranded polynucleotides are removed preferably by adding a thermolabile single-stranded nuclease to the sample under conditions and for a time sufficient to digest single-stranded polynucleotides in the sample, and then heating the sample to inactivate the single-stranded nuclease. In (M2), the first TP is at least one allele of a locus comprising a second allele, and where the first primer preferentially hybridizes to and is extended from the at least one allele as compared to second allele, where first and second primers has at least one mismatch with the second allele at one of the five 3' nucleotide of the first primer, and at least one allele and the second allele differ by a single nucleotide polymorphism. The method further comprises contacting the sample with a flanking primer has a lower melting point for hybridization to the first target polynucleotide than the first primer that is

complementary to the first target polynucleotide and can be extended to form a flanking primer extension product, where the flanking primer is complementary to the first target polynucleotide at a flanking position that is 5' to a position at which the first target complementary region is complementary to the first target polynucleotide. (M2) further comprises forming a second AMP detection complex for assaying the sample for second TP. Utilizing a third and a fourth primer. Preferred Kit: The label is provided conjugated to a nucleotide which can be incorporated into the AMP, The label is provided conjugated to the second primer. the substrate is attached to a plurality of different **capture** probes, where each of said different **capture** probes is attached at an identifiable location on the substrate, where each of said different **capture** probes can preferentially hybridize to a corresponding different AMP, each of the corresponding different AMPs comprising a label that can be the same or different as the label on the other corresponding different AMPs and where the instructions further describe how to use the components to the kit to assay the sample for each of the corresponding different AMPs. M2 comprises: (a) providing a first and second primer; where first primer comprising a 3' end a first complementary region that is complementary to the first polynucleotide, first complementary region located at the 3'- end of the first primer, and a first target non complementary region that is not complementary to the first TP at a position at 3' of a sequence to which the first target complementary region can hybridize, the first primer comprising a 3'-end and a first label; (b) providing the sample the sample suspected of containing the first TP; (c) contacting the sample with the first primer under conditions in which the first target complementary region can hybridize to the first TP and the first primer can be extended to form a first primer extension product; (d) altering the sample condition to allow disassociation of the first primer extension product from the first target polynucleotide, where the 3'-end of the first primer is complementary to the first primer extension product at a position in the first extension product that is 3' to the first target complementary region; (e) contacting the sample with the first primer under conditions in which the first can hybridize to the first primer extension product and be extended to form a first extension product comprising a first **capture** sequence that is the complement of the first target non complementary region and does not exist elsewhere in the first primer extension product, where the first primer extension product is the AMP; (f) altering the sample condition to allow dissociation of the first primer extension product from the first extension product; and (g) contacting the sample with a first **capture** probe conjugated to a first substrate where the contacting takes place under conditions in which the first **capture** probe can bind to the first **capture** sequence of the second primer extension product to form an AMP detection complex.

USE - (M2) is useful for forming an AMP detection complex for assaying a sample for first TP. The method further comprises determining if the first label is associated with the first substrate, where AMP is produced at a detectably higher level from at least one allele of a locus having at least two alleles and the first substrate preferably a first microsphere comprising a first spectral code is identified by decoding the spectral code which is preferably performed prior to, simultaneously or subsequent to determining if the first label from the second primer is associated with a substrate, first TP, preferably single-stranded or double-stranded **DNA** or RNA and a polymerase, preferably **DNA** polymerase having reverse transcriptase activity is used to form the first primer extension product (claimed). (M1) is useful for particular polynucleotide sequences, whether based on SNPs, conserved sequences, or other features or useful in a wide variety of different applications. The method is useful for pharmacogenetic testing, such methods ca be used in a forensic setting to identify the species or individual which was the source of a forensic specimen. Polynucleotide analysis methods can also be used in an anthropological setting.

Paternity testing is another area, as is testing for compatibility, between prospective tissue or blood donors and patients in need, and in screening for heredity disorders. (M1) is also useful for studying gene expression in response to a stimulus. Other applications include human population genetics, analyses of human evolutionary history, and characterization of human haplotype diversity. The method is useful to detect immunoglobulin class switching and hypervariable mutation of immunoglobulins, to detect polynucleotide sequences from contaminants or pathogens including bacteria, yeast, viruses, for HIV subtyping to determine the particular strains or relative amounts of particular strains infecting an individual, and can be repeatedly to monitor changes in the individuals predominant HIV strains, such as the development of drug resistance or T cell tropism; and to detect single nucleotide polymorphisms, which may be associated with particular alleles or subsets of allele. Over 1.4 million different single nucleotide polymorphisms (SNPs) in the human population have been identified. The method is also useful for mini-sequencing and for detection of mutations. Any type of mutation can be detected, including without limitation SNPs, insertions, deletions, transitions, transversions, inversions, frame shifts, triplet repeat expansions, and chromosome rearrangements. The method is useful to detect nucleotide sequences associated with increased risk of diseases or disorders, including cystic fibrosis, Tay-Sachs, sickle-cell anemia, etc. The method is useful for any assay in which a sample can be interrogated regarding an amplification product from a target polynucleotide. Typical assays involve determine the presence of the amplification product in the sample or its relative amount, or the assays may quantitative or semi-quantitative. Results from such assays can be used to determine the presence or amount of the target polynucleotide present in the sample. The above methods are particularly useful in multiplex settings where several TP are to be assayed.

EXAMPLE - 80 mul of 10 micron polysciences beads were spun down per conjugation reaction. The supernatant was removed and the beads were resuspended in 100 mul pf imidazole buffer. The beads were spun down again and the supernatant removed. The beads were again washed in imidazole buffer. After the second wash, the beads were resuspended in 20 mul imidazole buffer. 2.0 mul of 100 muM oligonucleotide and 100 mul of 200 mM 1-(3-(dimethyl-amino)-propyl)-ethylcarbodiimide hydrochloride (EDC) made fresh imidazole buffer pH 7.0 were added to the beads. The beads were then incubated for 4 hours at room temperature with shaking. The beads were then spun down, the supernatant removed, and the beads were resuspended. This step was then repeated. The beads were then again spun down, the supernatant was removed and the beads were resuspended in 100 mul of water, this step was also repeated. The beads were then spun down, the supernatant removed and the beads were resuspended in 45 mul of PBS pH 7.4. An amplification reaction was carried out using IX Amplitaq Stoffel buffer (10 mM Tris-HCl, 10 mM KCl, pH 8.3, 200 muM each dNTP), 25 mM Magnesium chloride, 2.5 U Amplitaq Stoffel enzyme, 200 mM **reverse primer** (5'-biotin-TTCAGTGCCAACCGCCTCAC-3'), 100 nM **forward primer** (5'-GCAATAGGTTTTGAGGGGCATggttgtggaa gaggac-3'), 100 nm **forward primer** (5'-TTCTGGGCCACTGACTGATTTggttgtggaagagaac-3') (where target noncomplementary regions are in capital letters and target-specific region of the primer are in lowercase letters), 1 ng/mul genomic **DNA**. The heat denatured amplicon was added to 2.5 mul of conjugated beads in 85 mul of hybridization buffer. The beads was spun down, by supernatant removed and then resuspended in 100 mul 1.5XSSPE (225 mM NaCl), 0.1% sodium dodecyl sulfate. The bead were again spun down and the supernatant removed to wash away excess amplicon. The beads were then resuspended in 50 mul of PBS/BSA. 0.5 mul of 0.2 mg/ml **streptavidin**-Cy-Chrome dye was added and incubated with the beads for 1 hour at room temperature. The beads were then spun down and resuspended in 400 mul PBS pH 7.4. The beads were analyzed on flow cytometer in PBS pH 7.4. The unique **capture** sequence-tagged amplicon showed sequence-specific hybridization to its complement

attached to beads. Both alleles present in the heterozygous sample could be independently amplified and identified, and heterozygous samples could be distinguished from homozygous samples. (85 pages)

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L11 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:162310 CAPLUS
DOCUMENT NUMBER: 140:176229
TITLE: Diagnostic polymerase chain reaction process utilizing simultaneous **capture** and detection of amplicons
INVENTOR(S): Hofmann, Scott Daniel
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 4 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004038194	A1	20040226	US 2001-974648	20011009

PRIORITY APPLN. INFO.: US 2000-238792P P 20001006
AB A method simultaneously detects and **captures** double-stranded **DNA** (dsDNA) sequence. The method includes adding a **forward primer** and a **reverse primer** for the dsDNA sequence in a sample; either the **forward primer** or the **reverse primer** have a **capture** agent, and the other has a detection agent. Replication of the dsDNA by PCR results in a segment of dsDNA with one specific end designed to be **captured** onto a medium and the opposite end designed for detection. The method is intrinsic and simultaneous and lends itself to ease of purification after **capture** and prior to detection and subsequent quantification. The method also can be applied to dsDNA that has been reverse -transcribed from single-stranded RNA. This procedure is exemplified by detecting an HIV viral load.

L11 ANSWER 2 OF 9 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-13777 BIOTECHDS
TITLE: Determining the genetic haplotype of a part of an individual genotype, comprises determining the presence or absence of specific nucleotide polymorphisms of a pair of homologous chromosomes;
with use of polymerase chain reaction and ligase chain reaction
AUTHOR: FENGER M; BENTZEN J
PATENT ASSIGNEE: HVIDOVRE HOSPITAL
PATENT INFO: WO 2003018835 6 Mar 2003
APPLICATION INFO: WO 2002-DK552 22 Aug 2002
PRIORITY INFO: DK 2001-1252 23 Aug 2001; DK 2001-1252 23 Aug 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-342453 [32]
AN 2003-13777 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Detecting the presence/absence of multiple haplotypic genetic variations in a preselected region of one chromosome of a chromosome pair comprises selecting nucleotide polymorphisms, suspected to occur in the preselected region, isolating a target **nucleic acid** (TNA) of the chromosome pair from a sample, and detecting presence/absence of several polymorphisms, using designed oligonucleotide

probes.

DETAILED DESCRIPTION - Detecting the presence/absence of multiple haplotypic genetic variations in a preselected region of one chromosome of a chromosome pair comprises: (a) selecting several nucleotide polymorphisms suspended to occur in the preselected region; (b) designing a set of oligonucleotide probe members, each of which is capable of hybridizing specifically with one individually selected nucleotide sequence in the preselected region comprising at least one polymorphism suspected to occur in the preselected region; (c) obtaining from an individual a **nucleic acid** sample which contains a target **nucleic acid** comprising the preselected region of the one member or a chromosome pair; (d) isolating or specifically analyzing at least part of the target **nucleic acid** of the one member of a chromosome pair comprising the preselected region, thus obtaining a resolution of the haplotypes from the individual; and (e) detecting the presence or absence of several polymorphisms in the preselected region by **nucleic acid** hybridization with the set of oligonucleotide probe members. An INDEPENDENT CLAIM is also included for a kit for detecting the presence or absence of multiple haplotypic genetic variation in a preselected region of one chromosome of a chromosome pair.

BIOTECHNOLOGY - Preferred Method: One probe of the set is an allele-specific **capture** probe or primer, which is specific for one allele of a first polymorphism in the preselected region, and the other probe of the set is an allele specific detection-probe which is specific for one allele of a second polymorphism in the preselected region. The method further comprises isolating at least a part of the target **nucleic acid** sequence by **capturing** the target **nucleic acid** sequence with the allele specific **capture** probe, or isolating the target **nucleic acid** comprises amplifying it using a primer pair of which one member is the allele specific primer, and obtaining a resolution of the haplotypes, where the probe is differently labeled. The method further comprises detecting the presence or absence of several polymorphisms by contacting the amplified target **nucleic acid** with the allele specific detection probe, and detecting the hybrid between the target **nucleic acid** and the allele specific detection probe. Further, the method comprises providing **capture** probes specific for every allele of a first polymorphism, where the **capture** probes are immobilized in different positions to enable the resolution of both haplotypes of a diploid individual, or the amplification of every allele of a first polymorphism to separate amplification reactions to enable resolution of both haplotypes of a diploid individual. The method comprises specifically analyzing the target **nucleic acid** by contacting the target with the multifunctional detection probe, and obtaining a resolution of the haplotypes, where the multifunctional detection probes can detect every combination of alleles for the multiple polymorphisms. The target **nucleic acid** is selected from genomic **DNA**, cDNA, mRNA, mitochondrial **DNA** and chloroplast **DNA**. At least one member of the set of oligonucleotide probes are labeled with a fluorescent reporter group (fluorescein, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, acridin, Hoechst 33258, rhodamine, rhodamine green, tetramethylrhodamine, Texas red, cascade blue, Oregon green, Alexa flour, europium, and samarium), with an enzyme tag (beta-galactosidase, peroxidase, horseradish peroxidase, urease, glycosidase, alkaline phosphatase, chloramphenicol acetyltransferase and luciferase), with a chemiluminescent group (luminol and oxalate esters), or with a radioisotope (hydrazides and the radioisotope is selected from 32P, 32P, 35S, 125I, 45Ca, 14C and 3H). Isolation of the resolved haplotype comprises the binding of at least one member of the set of oligonucleotide probes to a solid surface, where the preselected region is amplified, preferably by an allele specific amplification method. The amplification is performed by PCR, Ligase Chain Reaction (LCR),

Nucleic Acid Sequence-Based Amplification (NASBA), strand displacement amplification, rolling circle amplification, and T7-polymerase amplification. The method further comprises the use of an allele-specific primer coupled to an entity suitable for a subsequent immobilization reaction, performing the amplification reaction, and thus obtaining an amplified **DNA** molecule with the entity coupled to one of the **DNA** strands, dissociating the two strands of the amplified **DNA** molecule, binding only the coupled **DNA** strand to a solid surface, and contacting the bound **DNA** with several differently labeled oligonucleotide probes each of which is capable of detecting one specific polymorphism by **nucleic acid** hybridization. The solid surface is selected from nitrocellulose surface, a **cellulose** surface, a diazotized surface and a nylon surface. Binding further comprises coupling one member of an affinity pair to the **nucleic acid** while the other member of the affinity pair is immobilized on the solid surface. The affinity pair is selected from biotin-**streptavidin**, biotin-**avidin**, digoxigenin-anti-hapten antibody, fluorescein-anti-hapten antibody, lectins-lectin receptor, ion-ion chelators, immunoglobulin (Ig) G-protein A, IgG-protein G and magnets-paramagnetic particles. Binding comprises the coupling of the **nucleic acid** to a photoreactive group, or chemoreactive group. The photoreactive group is selected from quinone, an anthraquinone, psoralene, coumarin, benzofuran, indole and a photoactivable ketone, including benzophenone and acetophenone. The chemoreactive group is selected from sulfhydryl, a primary amine and a primary phosphate. The resolution of haplotypes of a preselected region of the one member of a chromosome pair is performed by hybridization techniques in a one-phase system. Several nucleotide polymorphisms of the preselected region is detected by hybridization in suspension with one labeled oligonucleotide probe which is capable of detecting several polymorphisms, such as at least 2 polymorphisms, for e.g., up to 10 polymorphisms. Several nucleotide polymorphisms are detected by hybridization in suspension with several different labeled oligonucleotide probes each of which is capable of detecting one specific polymorphism. The preselected region is amplified by the above mentioned allele specific amplification methods. In the method, the spacer comprises 8-28 nucleotides, where two adjacent polymorphisms are separated by at least 15- 150 nucleotides, preferably at least 200 nucleotides, for e.g., at least 250 nucleotides, or more preferably up to 1000 kb. Preferred Kit: The kit comprises: (a) at least two oligonucleotide probes, each of which is capable of hybridizing specifically with one allele of an individually selected polymorphic locus occurring in the preselected region, one of the probe is a detection probe, which is bound to a label, two adjacent polymorphic loci is separated by at least 15 nucleotides; and (b) buffers and solutions for carrying out hybridization under conditions of high stringency between the oligonucleotide probes and a target **nucleic acid** sequence comprising the preselected region. One probe is a **capture** probe coupled to an entity suitable for a subsequent immobilization reaction and the other probe is a detection probe. The kit comprises a primer pair, one of which is an allele specific primer, which is an oligonucleotide probe, and an allele specific probes to resolve all possible combinations of allele for the two or more polymorphisms. The kit further comprises multifunctional oligonucleotide probe comprising: (a) a first allele specific nucleotide sequence, which is specific for one allele of a first polymorphic locus; (b) a second allele specific nucleotide sequence, which is specific for one allele of a second polymorphic locus; (c) a spacer sequence, which does not hybridize to any part of the target **nucleic acid** under conditions where the allele specific sequences hybridize, and which separates the first and second allele specific nucleotide sequences; and (d) a detectable label.

USE - The method is useful for detecting the presence or absence of

multiple haplotypic genetic variations in a preselected region of one chromosome of a chromosome pair (claimed). The method is also useful for determining the linkage phase between two adjacent loci.

ADVANTAGE - The method is fast, inexpensive and provides uncomplicated determination of haplotypes in **nucleic acid** fragments of varying length from 25-40000 base pairs.

EXAMPLE - Detection of ApoB haplotype by hybridization to an immobilized detection probe was as follows. Human genomic **DNA** was isolated from 5 ml mammalian full blood, two primers, **forward primer**: 5'-GATGAAACCAATGACAAAATCC-3', and **reverse primer**: 5'-TGCTCCGTTCTCAGGTACTTGC-3' spanning the region of the 2488 and 2712 polymorphisms were designed and PCR amplification was carried out. Two oligonucleotides, 21 base pairs long, biotinylated in the 5' and which were complementary to either the C-allele or the T-allele of the rodon 2488 polymorphisms were designed. These oligos were attached to a microtiter plate well coated with **streptavidin**. The two oligos were placed on separate surfaces (e.g. the one complementary to the C-allele in one microtiter plate well and the one complementary to the T-allele in a different well). The PCR amplicon was added to the oligonucleotides attached to the solid surface and allowed to hybridize. The surfaces were shaken and then washed with 200 microl 1xSSCT buffer 5 times. The PCR-amplicons, which do not contain a perfect match for the attached oligonucleotide would not have hybridized and was therefore washed off, while the PCR-amplicons, which contained a sequence 100% complementary to the one possessed by the oligonucleotide was hybridized. (56 pages)

L11 ANSWER 3 OF 9 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-10572 BIOTECHDS
TITLE: New dye-labeled ribonucleotide triphosphate analogs useful for **DNA** sequencing by polymerase chain reaction; plasmid **DNA** sequencing using polymerase chain reaction, also useful for SNP detection and genomics analysis
AUTHOR: FISHER P V; VATTA P; KHAN S H
PATENT ASSIGNEE: PE CORP NY
PATENT INFO: WO 2003000841 3 Jan 2003
APPLICATION INFO: WO 2002-US16587 21 Jun 2002
PRIORITY INFO: US 2001-886011 22 Jun 2001; US 2001-886011 22 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-229331 [22]
AN 2003-10572 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Dye-labeled ribonucleotide triphosphate analogs, are new.

DETAILED DESCRIPTION - Dye-labeled ribonucleotide triphosphate analogs of formula (I) are new. B' = nucleobase; L = linker; R3 = triphosphate, alpha-thiotriphosphate or its salt; and T = reporter group. INDEPENDENT CLAIMS are also included for: (1) determining the sequence of a **DNA** template comprising: (a) annealing at least one oligonucleotide primer to a template; (b) incubating the oligonucleotide primer with a **DNA** polymerase that can incorporate both deoxynucleotides (dNTPs) in a reaction comprising a mixture (a1) of unlabeled dNTPs and at least one dye-labeled ribonucleotide to form primer extension products; (c) treating the primer extension products with a device (A) for hydrolyzing the extension products at each occurrence of a ribonucleotide; (d) separating the resulting fragments that contain the at least one primer from other fragments; (e) resolving the primer-containing extension product by size; and (f) detecting the fragments; (2) detecting mutations in **DNA** comprising: (a) annealing two oligonucleotide primers to a template; (b) incubating the two oligonucleotide primers with a **DNA** polymerase that can incorporate both dNTPs in a reaction comprising (a) to form primer extension products; (c) treating the primer extension products with (A)

to produce fragments; (d) resolving the fragments by size; and (e) detecting the fragments; (3) preparation of polynucleotide fragments comprising: (a) incubating the **DNA** template with the **DNA** polymerase, dATP, dGTP, dCTP, dTTP, at least two oligonucleotide primers complementary to the **DNA** template and (I) so that the primers are extended and the dye-labeled ribonucleotide is incorporated in the primer extension products and hydrolyzing 3'-5'-phoshphodiester linkages between adjacent ribo- and deoxyribonucleotides; (4) preparation of dye-labeled RNA complementary to a sequence of interest comprising preparing a mixture of a template, RNA polymerase, rATP, rGTP, rCTP, rUTP and at least one (I) oligonucleotide primers complementary to the **DNA** template (the sequence of interest is operable linked to a site for the initiation of RNA synthesis by the RNA polymerase), and incubating the mixture so that the RNA polymerase catalyzes the synthesis of RNA; and (5) detection 5-methylcytosine in the **DNA**-template comprising: (a) treating the template with a bisulfite salt such that 5-methylcytosine remains non-reactive; (b) incubating the **DNA** template with the **DNA** polymerase, dATP, dGTP, dCTP, dTTP, at least two oligonucleotide primers complementary to the **DNA** template and a dye-labeled rCTP compound so that the primers are extended and the dye-labeled rCTP compound is incorporated in the primer extension products; (c) hydrolyzing 3'-5'-phoshphodiester linkages between adjacent ribo- and deoxyribonucleotides to produce fragments; resolving the fragments by size and detecting the fragments.

USE - For determining the sequence of a **DNA** template, for detecting mutations (e.g. single nucleotide polymorphism) in **DNA** (e.g. genomic **DNA**) and for detection of 5-methylcytosine in the **DNA** template, and for preparing dye-labeled RNA complementary to a sequence of interest (all claimed). As hybridization probes and in the synthesis of dye-labeled RNAs which are useful in quantifying the yield from an in vitro RNA synthesis and for preparing antisense and/or sense probes for in situ hybridization. Also for direct PCR sequencing.

ADVANTAGE - The compounds are efficiently incorporated into primer extension products by modified thermostable **DNA** polymerase.

EXAMPLE - A reaction mixture (20 microliters) comprised of 25 mM Tris-Cl, pH 8.8 at 20 degrees C, 1 mM beta-mercaptoethanol, 50 mM KCl, 1.25 MeCl2, 100 microM dATP, 100 microM dCTP, 100 microM dGTP, 100 microM dUTP, biotinylated **forward primer** (200 nM), **reverse primer** (200 nM), 50 nM rUTP-propargylamine-6-TAMRA (684 microM in 50 mM CAPSO (95 microliters)), 50 nM rCTP-propargylamine-6-Rox (rhodamine X) (162 microM in 50 mM CAPSO (115 microliters)), 50 nM rGTP-propargylamine-5-R110 (rhodamine 110), 25 nM rATP-propargylamine-6-R6G (505 microM in 50 mM CAPSO (95 microliters)), Tma polymerase 25 R (0.75 units), 30R Tma polymerase (3 units), Taq polymerase (2.5 units) and template **DNA** (pGEM 3Zf(+)) (6 ng). The reaction mixture was placed in a thermal cycle, heated to 95 degrees C for 45 seconds and then subjected to 45 cycles of 95 degrees C for 15 seconds, 55 degrees C for 30 seconds and 65 degrees C for 3 minutes. The final incubation was continues at 65 degrees C for an additional 10 minutes. 1 microliters of the mixture was analyzed on a 2 % agarose gel to confirm that the amplicon was a unique band. The remaining mixture was mixed with 250 mM EDTA (2 microM) and 1 N NaOH (10 microliters) and heated to 98 degrees C for 10 minutes to hydrolyze the primer extension products at sited of dye-labeled rNTP incorporation. The solution was cooled and neutralized by the addition of 1N HCl (10 microliters). Fragments containing the **forward primer** were **captured** on **avidin**-coupled magnetized beads and washed. The **captured** fragments were eluted from the beads by heating to 98 degrees C for 2 minutes in loading buffer and analyzed on **DNA** sequencer. The sequence determined, which extended through the complete multicloning site between the forward and reverse primers base pairs from the **forward primer**, matched the known sequence of pGEM 3Zf(+). (48 pages)

L11 ANSWER 4 OF 9 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-00777 BIOTECHDS
TITLE: New isolated melanocortin-5 receptor useful for identifying a compound that binds to melanocortin-5 receptor and identifying antagonists/agonists of canine melanocortin-5 receptor useful for treating skin disorders; recombinant protein production and antagonist and agonist for use in disease therapy and gene therapy
AUTHOR: HOUSEKNECHT K L; ROBERTSON A S; XIAO Y
PATENT ASSIGNEE: HOUSEKNECHT K L; ROBERTSON A S; XIAO Y
PATENT INFO: US 2003110518 12 Jun 2003
APPLICATION INFO: US 2002-256089 25 Sep 2002
PRIORITY INFO: US 2002-256089 25 Sep 2002; US 2001-325646 28 Sep 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-829407 [77]
AN 2004-00777 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated melanocortin-5 receptor (MC5R) polypeptide comprising an amino acid sequence chosen from a fully defined sequence of 325 amino acids, as given in the specification, an amino acid sequence encoded by canine MC5R clone, and an extracellular or cytoplasmic domain of canine MC5R or of polypeptide encoded by canine MC5R clone, is new.

DETAILED DESCRIPTION - An isolated canine melanocortin-5 receptor (MC5R) polypeptide (I) comprising an amino acid sequence chosen from a fully defined sequence of 325 amino acids (S1) as given in the specification, an amino acid sequence encoded by canine MC5R clone, an extracellular domain of a canine MC5R corresponding to amino acids 1-37, 90-119, 181-185, or 265-272 of (S1) or of polypeptide encoded by canine MC5R clone, and a cytoplasmic domain of a canine MC5R corresponding to amino acids 63-70, 139-160, 213-237, or 297-325 of (S1) or of polypeptide encoded by canine MC5R clone deposited with the ATCC having ATCC Accession Number PTA-3455 (A1). INDEPENDENT CLAIMS are also included for: (1) an isolated **nucleic acid** (II) encoding a functional MC5R, or its complement, the **nucleic acid** comprising a nucleotide sequence chosen from a nucleotide sequence which hybridizes under conditions of moderate stringency to the coding region of a fully defined sequence of 3945 nucleotides (S2) as given in the specification, a nucleotide sequence which hybridizes under conditions of moderate stringency to a polynucleotide which is complementary to the coding region of (S2), a nucleotide sequence which hybridizes under conditions of moderate stringency to the coding region of the canine MC5R as deposited with (A1), and a nucleotide sequence which hybridizes under conditions of moderate stringency to a polynucleotide which is complementary to the coding region of the canine MC5R as deposited with (A1), provided that the functional MC5R is not human, bovine, chimpanzee, murine, rat, chicken or swine; (2) an isolated **nucleic acid** encoding a functional MC5R, or its complement, the **nucleic acid** comprising a nucleotide sequence chosen from a nucleotide sequence which hybridizes under conditions of high stringency to (S2), a nucleotide sequence which hybridizes under conditions of high stringency to a polynucleotide which is complementary to the coding region of (S2), a nucleotide sequence which hybridizes under conditions of high stringency to the coding region of the canine MC5R as deposited with (A1), and a nucleotide sequence which hybridizes under conditions of high stringency to a polynucleotide which is complementary to the coding region of the canine MC5R as deposited with (A1); (3) an isolated **nucleic acid** (III) comprising a nucleotide sequence that encodes a polypeptide having (S1), or encodes a polypeptide encoded by the canine MC5R clone as deposited with (A1); (4) an isolated **nucleic acid** comprising a nucleotide sequence having more than 87 % identity to (S1) or a fully defined sequence of 978 nucleotides (S3) as given in the specification or to the

canine MC5R clone as deposited with (A1); (5) an isolated **nucleic acid** comprising a nucleotide sequence encoding an extracellular domain of a canine MC5R corresponding to amino acids 1-37, 90-119, 181-185, or 265-272 of (S1) or of the polypeptide encoded by the canine MC5R clone as deposited with (A1); (6) an isolated **nucleic acid** comprising a nucleotide sequence encoding a cytoplasmic domain of a canine MC5R corresponding to amino acids 63-70, 139-160, 213-217, or 297-325 of (S1) or of the polypeptide encoded by the canine MC5R clone as deposited with (A1); (7) a nucleotide vector (IV) comprising (II) under the control of a nucleotide regulatory element with which the **nucleic acid** is not naturally associated; (8) a genetically engineered host cell (V) that expresses functional MC5R and comprises (II); (9) an isolated polypeptide (VI) encoded by (II); (10) identifying a compound that binds to (VI); (11) an antibody (VII) that immuno-specifically binds a polypeptide having (S1); (12) producing (I); (13) detecting a polynucleotide comprising (II); (14) identifying (ant)agonists of canine MC5R; (15) preparing recombinant canine MC5R polypeptide; (16) a composition (VIII) comprising a substantially pure polypeptide having (S1); (17) a transgenic animal, the nucleated cells of which comprise a transgene encoding (VI); and (18) a composition (IX) comprising an agonist or antagonist of canine MC5R identified by (I) and a carrier.

BIOTECHNOLOGY - Preparation (claimed): (I) is prepared by culturing a host cell transformed with an expression vector which expresses the recombinant canine MC5R polypeptide in the host cell membranes, isolating membranes having (I) from the host cell and recovering the recombinant canine MC5R polypeptide from the cell culture. Preferred **Nucleic Acid**: In (III), the **nucleic acid** has (S2), (S3) or the canine MC5R clone as deposited with (A1). Preferred Host Cell: In (V), the **nucleic acid** is in operative association with a nucleotide regulatory element that controls expression of the nucleotide sequence in the host cell. Preferred Method: Detecting a polynucleotide comprising (II) in a sample comprises contacting the sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, the polynucleotide is detected. (II) is useful for detecting a polynucleotide comprising (II) in a sample comprising contacting the sample under stringent hybridization conditions with **nucleic acid** primers that anneal to the **nucleic acid** under such conditions, and amplifying the annealed polynucleotides, so that if a polynucleotide is amplified, the polynucleotide is detected. The polynucleotide is an RNA molecule that encodes a functional MC5R, and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide. Identifying a compound that binds to (VI) comprises contacting a compound with (VI) for a time sufficient to form a polypeptide/compound complex, and determining whether the polypeptide/compound complex is formed, where if the polypeptide/compound complex is formed, then a compound that binds to the polypeptide is identified. Alternatively, the method comprises contacting a compound with the polypeptide in a cell, for a time sufficient to form a polypeptide/compound complex, where the complex drives expression of a reporter gene sequence in the cell, and detecting the complex by detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds to the polypeptide is identified. Identifying antagonists of canine MC5R comprises contacting a cell line that expresses (VI) with a test compound in the presence of an MC5R agonist, and determining whether the test compound inhibits binding of the MC5R agonist to the MC5R or inhibits functional activity of the MC5R, in which antagonists are identified as those compounds that inhibit binding of the MC5R agonist to the MC5R or inhibit functional activity of the MC5R. Identifying agonists of canine MC5R comprises contacting a cell line that expresses (VI) with a test compound in the presence and in the absence of an MC5R agonist,

determining whether, in the presence of the MC5R agonist, the test compound inhibits the binding of the MC5R agonist to the cell line, and determining whether, in the absence of the MC5R agonist, the test compound mimics the cellular effects of the MC5R agonist on the cell line, in which agonists are identified as those test compounds that inhibit the binding but mimic the cellular effects of the MC5R agonist on the cell line.

ACTIVITY - Anabolic; Eating-Disorders-Gen.; Immunomodulator; Dermatological; Antiinflammatory; Antidiabetic; Anorectic; Antipruritic. No biological data given.

MECHANISM OF ACTION - Modulating appetite and/or metabolic rate. No biological data given.

USE - An isolated **nucleic acid** (II) encoding a functional MC5R is useful for detecting a polynucleotide comprising (II) in a sample. An isolated polypeptide (VI) encoded by (II) is useful for identifying a compound that binds to (VI). (VI) is useful for identifying (ant)agonists of canine MC5R. A composition (IX) comprising an agonist or antagonist of canine MC5R(IX) is useful for modulating the appetite and/or metabolic rate, or treating skin disorders of an animal in need of it which involves administering to the animal an MC5R ligand. The animal suffers from a decreased-appetite disorder and the MC5R ligand administered is an MC5R antagonist. The disorder is anorexia or cachexia. The animal suffers from type 2 diabetes or obesity and the MC5R ligand administered is an MC5R agonist. The skin disorder is seborrhea and the MC5R ligand administered is an antagonist. The skin disorder is pruritis or allergic dermatitis and the MC5R ligand administered is an MC5R agonist (all claimed).

ADMINISTRATION - A composition (IX) is administered by inhalation, oral, buccal, parenteral, topical, transdermal or rectal routes. No dosage given.

EXAMPLE - Canine skin tissue were prepared. cDNA libraries were prepared by Life Technologies. A biotinylated **capture** oligonucleotide had the sequence CATAGGGGCCATAGTGAAGAACAAAAACC which was designed from a conserved region of the isolated melanocortin-5 receptor (MC5R) polynucleotide sequence. Canine MC5R clones were isolated from their corresponding cDNA libraries using the oligonucleotide and the GENETRAPPER (RTM) system. Positive selection was achieved by stringently hybridizing the oligonucleotide to clones in a cDNA library. The complex was separated from all other clones in the library using **Streptavidin** magnetic beads, which were pelleted using a magnet. MC5R clones were identified by PCR using a MC5R specific primer pair designed to be outside of the **capture** oligonucleotide site (**forward primer**: TTCGCCGTGTGAAGAGATGG; **reverse primer**: ACGCCTCACGGTCATGATGT). One canine MC5R clone was isolated from the cDNA libraries, yielding a clone of 3.9 kilobases in size. Identity of the clones was confirmed by PCR using a different MC5R polynucleotide sequence-specific primer pair (**forward primer**: TGAGGCGTTCCAGAGTGTTAT, **reverse primer** : GGAGCCCAGCACACGAT). Sequencing of the canine MC5R polynucleotide was completed using one standard and seven primer walking reactions from both ends of each clone. Sequences of the MC5R polynucleotide fragments were assembled based on their overlapping regions. The nucleotide sequence encoding canine MC5R was depicted as a fully defined sequence of 3945 nucleotides as given in the specification. Conceptual translation of the open reading frame (ORF) of the nucleotide sequence revealed the predicted amino acid sequence of canine MC5R as a fully defined sequence of 325 amino acids as given in the specification. The open reading frame nucleotide sequence was depicted as a fully defined sequence of 978 nucleotides as given in the specification. (35 pages)

L11 ANSWER 5 OF 9 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-00844 BIOTECHDS
TITLE: Detecting if an organism is homozygous or heterozygous in a target sequence, by combining differential hybridization or

restriction endonuclease digestion with immobilized array technology or electrophoretic separation;
gene mutation detection in plant, mammal or human using **DNA** primer and **DNA** probe for disease therapy and propagation

AUTHOR: SIEMERING K
PATENT ASSIGNEE: MURDOCH CHILDRENS RES INST
PATENT INFO: WO 2002050305 27 Jun 2002
APPLICATION INFO: WO 2001-AU1643 20 Dec 2001
PRIORITY INFO: AU 2000-2214 20 Dec 2000; AU 2000-2214 20 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-583551 [62]
AN 2003-00844 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Determining (M) presence or absence of a homozygous or heterozygous change in one or more nucleotides within a target nucleotide sequence, using forward and reverse primers which are labeled with reporter molecules which provide separate identifiable signals, or unlabeled primers where detection is accomplished by hybridization of a probe labeled at its 5' and 3' termini, is new.

DETAILED DESCRIPTION - Determining (M) presence or absence of a homozygous or heterozygous change in one or more nucleotides within a target nucleotide sequence, comprising: (a) amplifying target nucleotide sequence using forward and reverse primers to produce an amplified product, where at least one of the primers is labeled with a reporter molecule capable of facilitating the provision of an identifiable signal which can be distinguished from another receptor molecule if both primers are labeled, and at least one primer and its complementary form comprises a complementary sequence to an oligonucleotide sequence anchored to a solid support, and the primers introduce, abolish or hybridize to a target site within the amplified product in the presence or absence of a change in one or more nucleotides, and subjecting the amplified product to detection unit; or (b) the method of: (i) amplifying the target nucleotide sequence using forward and reverse primers to produce an amplified product, where one primer comprises one or more chemically modified nucleotides, bases or phosphodiester bonds so that a nucleotide strand which extends from the primer is resistant to exonuclease activity and the other primer comprises a nucleotide sequences having sense and complementary sequences immobilized to a solid support, where the primers introduces or abolishes a restriction endonuclease site within the amplified product in the presence or absence of a change in one or more nucleotides; (ii) digesting the amplified product with an exonuclease to digest the strand not amplified by the primer comprising the exonuclease-resistant nucleotides, bases or phosphodiester linkages to generate a single-stranded **nucleic acid** molecule comprising the potential presence or absence of a restriction endonucleases site and a nucleotide sequence complementary to an oligonucleotide sequence immobilized to the solid support; (iii) hybridizing to the single stranded **nucleic acid** molecule a probe that contains complementary to the restriction site that may have been introduced to generate a partial double-stranded molecule, where the probe comprises two reporter molecules capable of facilitating the provision of identifiable signals which can be distinguished from each other; (iv) digesting the partially double-stranded molecule with the restriction endonuclease whose site has been potentially introduced or abolished in the amplified product and subjecting the digested molecule to conditions to permit annealing to a set of the immobilized oligonucleotides comprising oligonucleotides which are sense or complementary to a portion of the amplified sequence introduced by at least one primer; and (v) detecting the relative proportion of signal by the reporter molecules, where an equal proportion of different signals or the presence of only one signal represents a homozygous presence or absence of change in the target nucleotide sequence and the presence of a

differential signal represents a heterozygous presence or absence of the change in target nucleotide sequence. An INDEPENDENT CLAIM is also included for an assay device for determining the presence or absence of a nucleotide or group of nucleotides in a **nucleic acid** molecule, comprising an array of immobilized oligonucleotides each complementary to a nucleotide sequence within an amplified product digested by one or more restriction endonucleases and unit to screen for the hybridization of a target sequence to the immobilized oligonucleotide array.

BIOTECHNOLOGY - Preferred Method: In (Mi), the detection unit comprises detecting the relative proportion of signal or lack of signal by the reporter molecules. The target site within the amplified product is a restriction endonuclease site. One or more of the forward or reverse primers introduces a restriction endonuclease site within the amplified product. The solid support is glass or a polymer such as **cellulose**, nitrocellulose, ceramic material, polyacrylamide, nylon, polystyrene and its derivatives, polyvinylidene difluoride, methacrylate and its derivatives, polyvinylchloride and polypropylene, preferably glass. Two or more oligonucleotide sequences are anchored to the solid support in the form of an array. The restriction endonuclease site is recognized by restriction enzymes listed in the specification such as AatI, AatII, AauI, BaeI, BamHI, Cac8I, CbiI, DraIII, DsaI, Eco1301, EcoRI, Esp3I, FspI, GsuI, HphI, ItaI, Ksp22I, LspI, MseI, NarI, PacI, RsaI, SecI, TaqII, UbaDI, Van91I, XagI, Zsp2I, etc. The reporter molecule is chloramphenicol, colorless galactosidase, colorless glucuronide, luciferin, and green fluorescent protein. Alternatively, the differential restriction endonuclease digestion is assessed electrophoretically, where the pattern of electrophoretic separation and/or the pattern of reporter molecule signaling is indicative of homozygous or heterozygous presence or absence of change in the target sequence. Preferred Device: The device is packaged for sale and contains instructions for use.

USE - (M) is useful for determining the presence or absence of a homozygous or heterozygous change in one or more nucleotides within a target nucleotide sequence. The target sequence is in a eukaryotic cell such as a plant cell or a mammalian cell in particular a human cell, and the target sequence is associated with a disease condition comprising one or more known genetic mutations. The disease condition is adreno-leukodystrophy, atherosclerosis, gaucher disease, gyrate atrophy, juvenile onset diabetes, obesity, paroxysmal nocturnal hemoglobinuria, phenylketonuria, refsum disease, tangler disease and hemochromatosis conditions involving transporters, channels and pumps such as cystic fibrosis, deafness, diastrophic dysplasia, long-QT syndrome, Menkes syndrome, Pendred syndrome, polycystic kidney disease, sickle cell anemia, Wilson's disease and Zellweger syndrome, conditions involving signal transduction such as ataxia telangiectasia, baldness, Cockayne syndrome, glaucoma, tuberous sclerosis, Waardenburg syndrome and Werner syndrome, conditions involving the brain such as Alzheimer's disease, amyotrophic lateral sclerosis, Angleman syndrome, Charcot-Marie-Tooth disease, epilepsy, essential tremor, fragile X syndrome, Friedreich's ataxia, Huntington's disease, Niemann-Pick disease, Parkinson's disease, Prader-Willi syndrome, Rett syndrome, spinocerebellar atrophy and William's syndrome, and conditions involving the skeleton such as Duchenne muscular dystrophy, Ellis-van Creveld syndrome, Marfan syndrome and myotonic dystrophy. (All claimed). (M) is useful for screening for polymorphic variants in the genome of plants such as during the tissue culture stages of plant propagation. The ability to identify polymorphic variants in plants such as due to somaclonal variation prevents unnecessary resources being wasted on plants with undesired properties.

EXAMPLE - A mutation at nucleotide 35 in the connexin 26 gene was identified either in the homozygous or heterozygous state. The mutation was a deletion of a guanine at position 35. This mutation was referred to as 35DELTAG. Two primers were developed, each labeled with a different reporter molecule and at least one comprising a nucleotide sequence

matching and complementary to oligonucleotide sequences immobilized to a solid support. This sequence on the primer was referred to as a tag sequence. The primers comprised a reporter molecule alone or linked to a tag sequence having matching and complementary sequences immobilized to a solid support. One primer comprised a tag sequence linked to a nucleotide sequence complementary to a region flanking the 35DELTAG region for the **forward primer** and a region downstream of this location for the **reverse primer**. The **reverse primer** introduced a base change in the wild-type sequence thus creating a EcoRII site. If the target sequence comprised a 35 DELTAG mutation then the EcoRII site was lost, as EcoRII recognized the nucleotide sequence 5'CCWGG3', where W is A or T. In the connexin 26 gene, the nucleotide sequence recognized by EcoRII was 5'CCTGG3'. A 35 DELTAG mutation removed the G at the 3' position and hence, amplification product from a 35 DELTAG sample did not digest, while the wild-type sequence digested. After amplification and digestion with EcoRII, single-stranded forms of the amplified product were exposed to the immobilized oligonucleotides on the solid support. When the target sequence was homozygous wild-type, all the amplification product were digested thus removing the reporter molecule associated with the **reverse primer**. The complementary immobilized oligonucleotide (+) permitted **capture** of the tag associated with the **forward primer**. The matching (sense) immobilized oligonucleotide (-) permitted **capture** of the sequence complementary to the tag generated by extension of the **reverse primer** during polymerase chain reaction (PCR). As the reporter molecule associated with the **reverse primer** was cleaved away by EcoRII digestion, no reporter molecule was detected at the (-) feature of the immobilized oligonucleotide pair, i.e. the ratio of signal from forward to **reverse primer** was in the order of 1:0. When the 35 DELTAG mutation was present in a homozygous state, there was no digestion of any amplification product and both reporter molecules on both primers were equally represented, i.e. in a ratio of 1:1. When the 35DELTAG mutation was in the heterozygous state, then the amplification product from the nucleotide sequence carrying the mutation was not cleaved but cleavage occurred in the amplification product from the nucleotide sequence not carrying the mutation. About half of the molecules in the total amplification were cleaved. Accordingly, the ratio of signal from forward to **reverse primer** was 1:0.5. (73 pages)

L11 ANSWER 6 OF 9 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-04910 BIOTECHDS
TITLE: Analysis of polynucleotides in a sample using generic **capture** sequences comprises amplifying target polynucleotide, and utilizing the product to indirectly assay the sample for the polynucleotide;
DNA amplification useful for pharmacogenetics, forensics, anthropology, paternity testing, functional genomics, genetic analysis, SNP, immunoglobulin mutation, pathogen detection, drug resistance, **DNA** sequencing anddiagnosis
AUTHOR: LAI J H; PHILLIPS V E; WATSON A R
PATENT ASSIGNEE: QUANTUM DOT CORP
PATENT INFO: WO 2001083823 8 Nov 2001
APPLICATION INFO: WO 2000-US13979 28 Apr 2000
PRIORITY INFO: US 2000-200635 28 Apr 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-114152 [15]
AN 2002-04910 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Assaying, (M1) for an amplification product (AMP) from a first target polynucleotide, (TP), comprising providing a sample that is

suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a **capture** sequence not present in the TP at the same position, is new.

DETAILED DESCRIPTION - A target polynucleotide, (TP), is amplified, where first primer has a tag sequence, the complement of which is formed on the opposite strand during amplification and is referred to as **capture** sequence (CS), and the opposite strand is referred to as an amplification product (AMP) has a label; probe conjugated to substrate specific to CS, is contacted with AMP to form AMP detection complex. Assaying (M1) for an AMP from a first TP, comprises providing a sample that is suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a **capture** sequence not present in the TP at the same position, where the AMP is formed by primer extension from a template, where the template comprises a complement to the TP and a target noncomplementary region, where the **capture** sequence is a complement to the target noncomplementary region, providing a substrate that is conjugated to a first **capture** probe, contacting the sample with the **capture** probe under a first set of hybridization conditions, where the **capture** probe can bind to the **capture** sequence under the first set of hybridization conditions and determining if the first label is associated with the substrate. INDEPENDENT CLAIMS are also included for the following: (1) forming (M2) an AMP detection complex for assaying a sample for a first TP; (2) an amplification product detection complex comprising a **capture** probe polynucleotide hybridized to **capture** sequence of labeled amplification product from a TP, where the **capture** probe polynucleotide is conjugated to a substrate, where the **capture** sequence is not present in a region of the TP which is amplified and is introduced into the amplification product by copying a template polynucleotide, the template polynucleotide comprising a target noncomplementary region and a region complementary to the TP, where the target noncomplementary region was introduced into the template polynucleotide by extension of a primer hybridized to the target polynucleotide, the primer comprising the target noncomplementary region and where the **capture** sequence is complementary to the target noncomplementary region; and (3) a kit for assaying for an AMP from a TP comprises a substrate attached to a **capture** probe, a first primer comprising a 3' end a first target complementary region located at the 3' end of the first primer, and a first target noncomplementary region that is not complementary to the first TP at a position 3' of a sequence to which the first target complementary region can hybridize, a second primer, label, housing for retaining the substrate, first primer, second primer, and a label and instruction provided with the housing that describe how to use the components of the kit to assay a sample by forming an AMP from the TP using the first and second primers that comprises the label and a **capture** sequence that is complementary to the target noncomplementary in the first primer, where the **capture** sequence can bind to the **capture** probe.

BIOTECHNOLOGY - Preferred Method: In (M1) the **capture** probe is a polynucleotide, substrate is from micorsphere, chip, slide, multiwell plate, a membrane, an optical fiber, and an optionally porous gel matrix, more preferably slide. The substrate is preferably conjugated to several different **capture** probe polynucleotides having corresponding different sequences, where each of the different **capture** probes can selectively bind to a corresponding different **capture** sequence on a corresponding different AMP. The substrate is preferably a first microsphere comprising a first spectral code comprising a first semiconductor nanocrystal and first fluorescence characteristics, where the first semiconductor nanocrystal comprises: (a) a core selected from ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AIAs, AIP, AIS, Ge, Si, Pb, PObSe, PbSe, their alloy or their mixture, more preferably the core is a CdSe; (b) a shell, preferably ZnS. The AMP is produced by a process comprising

incorporation of a nucleotide comprising the first label into AMP using a polymerase or extension of a primer using a polymerase to form AMP, where primer comprises the first label, where the first label comprises an agent selected from chromophore; lumiphore; fluorophore preferably semiconductor nanocrystal, fluorescent dye, lanthanide chelate, a green fluorescent protein, more preferably is a lanthanide chelate selected from europium chelate, terbium chelate and a samarium chelate; chromogen; hapten; antigen; radioactive isotope; magnetic particle; metal nanoparticle; enzyme preferably alkaline phosphatase, horseradish peroxidase, beta-galactosidase, glucose oxidase, bacterial luciferase, an insect luciferase and sea pansy luciferase; antibody or binding portion or their equivalent; aptamer; and one member of a binding pair; or an agent selected from **avidin**, **streptavidin**, digoxigenin and biotin. The method preferably comprises determining if the first label, preferably a fluorophore, is associated with the substrate comprises applying a light source to the substrate that can excite the fluorophore, and determining if a fluorescence emission from the fluorophore occurs from the substrate. The sample is preferably assayed for the presence of AMP or to determine its amount, where AMP is produced at a detectably higher level from at least one allele of a locus having at least two alleles. When the substrate is a first microsphere, (M1) further comprises assaying the sample for containing a second, third and fourth AMP from a second, third and fourth TP, where second AMP from second TP and is further contacted under a second set of hybridization conditions with a second **capture** probe conjugated to a second microsphere, where the second **capture** probe is a polynucleotide, the second microsphere can be the first microsphere or a different second microsphere, the second microsphere is a different second microsphere comprising a second spectral code comprising second fluorescent characteristics, the second spectral code distinguishable from the first spectral code, the second **capture** probe can hybridize to the second AMP under the second set of hybridization conditions, the second AMP comprises a second label, which can be the first label when the second microsphere is a different second microsphere or can be a different second label, and determining if the second label is associated with the second microsphere, where the first and the second AMPs are produced from a single locus, or differ by a single nucleotide. The substrate is further conjugated to a second/third/fourth **capture** probe, where the second/third/fourth **capture** probe can preferentially bind to a second/third/fourth sequence on a second/third/fourth AMP, second/third/fourth comprising a second/third/fourth second/third/fourth label that can be the same as or different than the first label and/or the second label where the binding the second/third/fourth AMP to the second/third/fourth **capture** probe can be independently determined. In (M2), after altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, the sample is further contacted with the first and second primers under conditions in which the first and second primers can hybridize to the second and first primer extension products, respectively, and be extended to form several first and second primer extension products, where altering the sample conditions to allow dissociation of the first and second primer extension product from the first target polynucleotide and first primer extension product, respectively comprises heating the sample. (M2) further comprising removing single-stranded polynucleotides from the sample prior to altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, where removing single-stranded polynucleotides are removed preferably by adding a thermolabile single-stranded nuclease to the sample under conditions and for a time sufficient to digest single-stranded polynucleotides in the sample, and then heating the sample to inactivate the single-stranded nuclease. In (M2), the first TP is at least one allele of a locus comprising a second allele, and where the first primer preferentially hybridizes to and is extended from the at least one allele as compared to

second allele, where first and second primers has at least one mismatch with the second allele at one of the five 3' nucleotide of the first primer, and at least one allele and the second allele differ by a single nucleotide polymorphism. The method further comprises contacting the sample with a flanking primer has a lower melting point for hybridization to the first target polynucleotide than the first primer that is complementary to the first target polynucleotide and can be extended to form a flanking primer extension product, where the flanking primer is complementary to the first target polynucleotide at a flanking position that is 5' to a position at which the first target complementary region is complementary to the first target polynucleotide. (M2) further comprises forming a second AMP detection complex for assaying the sample for second TP. Utilizing a third and a fourth primer. Preferred Kit: The label is provided conjugated to a nucleotide which can be incorporated into the AMP, The label is provided conjugated to the second primer. the substrate is attached to a plurality of different **capture** probes, where each of said different **capture** probes is attached at an identifiable location on the substrate, where each of said different **capture** probes can preferentially hybridize to a corresponding different AMP, each of the corresponding different AMPs comprising a label that can be the same or different as the label on the other corresponding different AMPs and where the instructions further describe how to use the components to the kit to assay the sample for each of the corresponding different AMPs. M2 comprises: (a) providing a first and second primer; where first primer comprising a 3' end a first complementary region that is complementary to the first polynucleotide, first complementary region located at the 3'- end of the first primer, and a first target non complementary region that is not complementary to the first TP at a position at 3' of a sequence to which the first target complementary region can hybridize, the first primer comprising a 3'-end and a first label; (b) providing the sample the sample suspected of containing the first TP; (c) contacting the sample with the first primer under conditions in which the first target complementary region can hybridize to the first TP and the first primer can be extended to form a first primer extension product; (d) altering the sample condition to allow disassociation of the first primer extension product from the first target polynucleotide, where the 3'-end of the first primer is complementary to the first primer extension product at a position in the first extension product that is 3' to the first target complementary region; (e) contacting the sample with the first primer under conditions in which the first can hybridize to the first primer extension product and be extended to form a first extension product comprising a first **capture** sequence that is the complement of the first target non complementary region and does not exist elsewhere in the first primer extension product, where the first primer extension product is the AMP; (f) altering the sample condition to allow dissociation of the first primer extension product from the first extension product; and (g) contacting the sample with a first **capture** probe conjugated to a first substrate where the contacting takes place under conditions in which the first **capture** probe can bind to the first **capture** sequence of the second primer extension product to form an AMP detection complex.

USE - (M2) is useful for forming an AMP detection complex for assaying a sample for first TP. The method further comprises determining if the first label is associated with the first substrate, where AMP is produced at a detectably higher level from at least one allele of a locus having at least two alleles and the first substrate preferably a first microsphere comprising a first spectral code is identified by decoding the spectral code which is preferably performed prior to, simultaneously or subsequent to determining if the first label from the second primer is associated with a substrate, first TP, preferably single-stranded or double-stranded **DNA** or RNA and a polymerase, preferably **DNA** polymerase having reverse transcriptase activity is used to form the first primer extension product (claimed). (M1) is useful for

particular polynucleotide sequences, whether based on SNPs, conserved sequences, or other features or useful in a wide variety of different applications. The method is useful for pharmacogenetic testing, such methods ca be used in a forensic setting to identify the species or individual which was the source of a forensic specimen. Polynucleotide analysis methods can also be used in an anthropological setting. Paternity testing is another area, as is testing for compatibility, between prospective tissue or blood donors and patients in need, and in screening for heredity disorders. (M1) is also useful for studying gene expression in response to a stimulus. Other applications include human population genetics, analyses of human evolutionary history, and characterization of human haplotype diversity. The method is useful to detect immunoglobulin class switching and hypervariable mutation of immunoglobulins, to detect polynucleotide sequences from contaminants or pathogens including bacteria, yeast, viruses, for HIV subtyping to determine the particular strains or relative amounts of particular strains infecting an individual, and can be repeatedly to monitor changes in the individuals predominant HIV strains, such as the development of drug resistance or T cell tropism; and to detect single nucleotide polymorphisms, which may be associated with particular alleles or subsets of allele. Over 1.4 million different single nucleotide polymorphisms (SNPs) in the human population have been identified. The method is also useful for mini-sequencing and for detection of mutations. Any type of mutation can be detected, including without limitation SNPs, insertions, deletions, transitions, transversions, inversions, frame shifts, triplet repeat expansions, and chromosome rearrangements. The method is useful to detect nucleotide sequences associated with increased risk of diseases or disorders, including cystic fibrosis, Tay-Sachs, sickle-cell anemia, etc. The method is useful for any assay in which a sample can be interrogated regarding an amplification product from a target polynucleotide. Typical assays involve determine the presence of the amplification product in the sample or its relative amount, or the assays may quantitative or semi-quantitative. Results from such assays can be used to determine the presence or amount of the target polynucleotide present in the sample. The above methods are particularly useful in multiplex settings where several TP are to be assayed.

EXAMPLE - 80 mul of 10 micron polysciences beads were spun down per conjugation reaction. The supernatant was removed and the beads were resuspended in 100 mul pf imidazole buffer. The beads were spun down again and the supernatant removed. The beads were again washed in imidazole buffer. After the second wash, the beads were resuspended in 20 mul imidazole buffer. 2.0 mul of 100 muM oligonucleotide and 100 mul of 200 mM 1-(3-(dimethyl-amino)-propyl)-ethylcarbodiimide hydrochloride (EDC) made fresh imidazole buffer pH 7.0 were added to the beads. The beads were then incubated for 4 hours at room temperature with shaking. The beads were then spun down, the supernatant removed, and the beads were resuspended. This step was then repeated. The beads were then again spun down, the supernatant was removed and the beads were resuspended in 100 mul of water, this step was also repeated. The beads were then spun down, the supernatant removed and the beads were resuspended in 45 mul of PBS pH 7.4. An amplification reaction was carried out using IX Amplitaq Stoffel buffer (10 mM Tris-HCl, 10 mM KCl, pH 8.3, 200 muM each dNTP), 25 mM Magnesium chloride, 2.5 U Amplitaq Stoffel enzyme, 200 mM **reverse primer** (5'-biotin-TTCAGTGCCAACCGCCTCAC-3'), 100 nM **forward primer** (5'-GCAATAGGTTTTGAGGGGCATggttgtggaa gaggac-3'), 100 nm **forward primer** (5'-TTCTGGGCCACTGACTGATTTggttgtggaagagaac-3') (where target noncomplementary regions are in capital letters and target-specific region of the primer are in lowercase letters), 1 ng/mul genomic **DNA**. The heat denatured amplicon was added to 2.5 mul of conjugated beads in 85 mul of hybridization buffer. The beads was spun down, by supernatant removed and then resuspended in 100 mul 1.5XSSPE (225 mM NaCl), 0.1% sodium dodecyl sulfate. The bead were again spun down and the supernatant removed to wash away excess amplicon. The beads were

then resuspended in 50 mul of PBS/BSA. 0.5 mul of 0.2 mg/ml **streptavidin**-Cy-Chrome dye was added and incubated with the beads for 1 hour at room temperature. The beads were then spun down and resuspended in 400 mul PBS pH 7.4. The beads were analyzed on flow cytometer in PBS pH 7.4. The unique **capture** sequence-tagged amplicon showed sequence-specific hybridization to its complement attached to beads. Both alleles present in the heterozygous sample could be independently amplified and identified, and heterozygous samples could be distinguished from homozygous samples. (85 pages)

L11 ANSWER 7 OF 9 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1998-06397 BIOTECHDS
TITLE: Forward and reverse **DNA** sequencing in a single reaction;
application to plasmid and diagnostic **DNA** sequencing
AUTHOR: van den Boom D; Jurinke C; Ruppert A; *Koester H
CORPORATE SOURCE: Univ.Hamburg
LOCATION: Faculty of Chemistry, Department of Biochemistry and Molecular Biology, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany.
SOURCE: Anal.Biochem.; (1998) 256, 1, 127-29
CODEN: ANBCA2
ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1998-06397 BIOTECHDS
AB A new method for sequencing PCR products generates sense and antisense sequence information in one cycle sequencing reaction. The sequencing template is generated by an initial PCR with a biotinylated **forward primer** and a nonbiotinylated **reverse primer**. The unpurified product is subjected to dye terminator cycle sequencing with the same primers. The biotinylated forward sequencing products and reverse products are immobilized on **streptavidin**-coated magnetic beads. An additional PCR product containing the full-length biotinylated forward strand is added to **capture** the shortest reverse sequencing products. The immobilized strands are separated by denaturation using NH4OH at room temperature The nonbiotinylated reverse sequencing products are removed from the beads in this step. The biotinylated forward sequencing products are resolubilized with NH4OH at 60 deg. Both sequencing products are then run on an automated sequencer. Since crude PCR products can be sequenced directly in a single reaction, the method results in a reduced time and cost of the amplification and sequencing procedure. This method will be useful in plasmid and diagnostic **DNA** sequencing. (6 ref)

L11 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:189988 CAPLUS
DOCUMENT NUMBER: 126:182288
TITLE: Detection of amplified **nucleic acid** sequences using bifunctional haptenization and dyed microparticles
INVENTOR(S): Gerdes, John C.
PATENT ASSIGNEE(S): Immunological Associates of Denver, USA
SOURCE: PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9703207	A1	19970130	WO 1996-US11619	19960712

W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

US 5989813	A	19991123	US 1996-664863	19960617
CA 2226721	AA	19970130	CA 1996-2226721	19960712
AU 9664906	A1	19970210	AU 1996-64906	19960712
AU 715857	B2	20000210		
EP 837946	A1	19980429	EP 1996-924465	19960712

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

PRIORITY APPLN. INFO.: US 1995-2245P P 19950713
WO 1996-US11619 W 19960712

AB The invention describes an assay for detecting amplified target **nucleic acid** sequences with a visual signal defined by agglutination through the linking of microparticles with 2 distinct haptens, and alternatively, by linking microparticles to a **capture** zone on a lateral flow membrane or a filtration membrane with 2 distinct haptens. The sensitivity and specificity of the methodol. are based on bifunctional target labeling during the amplification step or subsequent hybridization that generates a bifunctional label. The method is illustrated by lateral flow chromatog. of bifunctionally labeled cytomegalovirus (CMV) amplification product. A **forward primer** carries a 5' digoxigenin label and a **reverse primer** carries a biotin 5' label, such that the sequence target for amplification of CMV is nucleotide 2758-3060. PCR amplification with biotin and digoxigenin yields a bifunctionally labeled amplicon, which is added to anti-digoxigenin coated microparticles and applied to a **streptavidin**-bound nitrocellulose membrane. The amplicon binds to the anti-digoxigenin microparticle wicks through the membrane to the **streptavidin** line and is **captured** by the interaction of biotin and **streptavidin**, resulting in a visible line of colored microparticles. The invention may be used, e.g., in the screening of amplicon detection for the purpose of more efficiently screening libraries. The invention is also useful to detect **nucleic acid** sequences indicative of a genetic defect or contagious disease when used with the appropriate primers, as well as detect the existence of **nucleic acid** amplification.

L11 ANSWER 9 OF 9 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 96090093 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7580847
TITLE: Quantitative analysis of lymphokine mRNA expression by an automated, non-radioactive method.
AUTHOR: Vandevyver C; Raus J
CORPORATE SOURCE: Department of Biotechnology, Dr. L. Willems-Instituut, Diepenbeek, Belgium.
SOURCE: Cellular and molecular biology (Noisy-le-Grand, France), (1995 Jul) 41 (5) 683-94.
Journal code: 9216789. ISSN: 0145-5680.
PUB. COUNTRY: France
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19960124
Entered Medline: 19951215

AB Variable gene expression, thus giving rise to variable mRNA levels, constitutes a major mechanism for controlling cell development and cell function. In order to investigate these changed mRNA levels, a sensitive and quantitative assay is required. A quick and easy method is described to quantify specific mRNA's by a combination of the polymerase chain reaction (PCR) and an electro-chemiluminescenct (ECL) detection of the amplified products. Total cellular RNA is reverse transcribed and amplified with a biotinylated **forward primer** and a TBR

(Tris (2,2'-bipyridine) ruthenium (II)) labelled **reverse primer**. The amplification product is **captured** on **streptavidin**-coated paramagnetic beads and quantified by ECL detection using the QPCR system 5000. The results can be converted to quantitative values with an external standard curve. In the present study, cytokine mRNA expression in T lymphocytes was quantified. Cytokine mRNA was measured at the attomolar range in a dynamic range up to three orders of magnitude. The ECL detection is quantitative, rapid and accurate.

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=> d his

     (FILE 'HOME' ENTERED AT 14:24:02 ON 04 JUN 2004)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 14:24:57 ON 04 JUN 2004
L1             88 S HOFMAN S?/AU
L2         205543 S DOUBLE STRANDED DNA OR DSDNA
L3             13 S L2 AND (FORWARD PRIMER AND REVERSE PRIMER)
L4              2 S L3 AND CAPTUR?
L5             13 DUP REM L3 (0 DUPLICATES REMOVED)
L6            735 S DNA AND (FORWARD PRIMER AND REVERSE PRIMER)
L7            394 S NUCLEIC ACID AND (FORWARD PRIMER AND REVERSE PRIMER)
L8             21 S (L6 OR L7) AND CAPTUR###
L9             11 S L8 AND (MALEAMIDE OR AVIDIN OR STREPTAVIDIN OR CELLULOSE)
L10             5 S L9 AND (IODINE-151 OR RADIOISTOPE OR FLUOROPHOR## OR FLUORES
L11             9 DUP REM L9 (2 DUPLICATES REMOVED)
L12             9 DUP REM L11 (0 DUPLICATES REMOVED)
L13             5 DUP REM L10 (0 DUPLICATES REMOVED)

=> s l1 and l6
L14             0 L1 AND L6

=> a l1 and l2
A IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s l1 and l2
L15             0 L1 AND L2

=> d l1 and DNA
'AND' IS NOT A VALID FORMAT
'DNA' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):end

=> s l1 and DNA
L16             6 L1 AND DNA

=> dup rem l16
PROCESSING COMPLETED FOR L16
L17             6 DUP REM L16 (0 DUPLICATES REMOVED)

=> d ibib abs l17

L17  ANSWER 1 OF 6  CAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2003:576088  CAPLUS
DOCUMENT NUMBER:          139:359543
```

TITLE: A linkage map for the hybridising toads Bombina bombina and B. variegata (Anura: Discoglossidae)
AUTHOR(S): Nuernberger, B.; **Hofman, S.**; Forg-Brey, B.; Praetzel, G.; Maclean, A.; Szymura, J. M.; Abbott, C. M.; Barton, N. H.
CORPORATE SOURCE: Department Biologie II, Ludwig-Maximilians-Universitaet, Munich, 80333, Germany
SOURCE: Heredity (2003), 91(2), 136-142
CODEN: HDTYAT; ISSN: 0018-067X
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Stable hybrid zones in which ecol. divergent taxa give rise to a range of recombinants are natural labs. in which the genetic basis of adaptation and reproductive isolation can be unraveled. One such hybrid zone is formed by the fire-bellied toads Bombina bombina and B. variegata (Anura: Discoglossidae). Adaptations to permanent and ephemeral breeding habitats, resp., have shaped numerous phenotypic differences between the taxa. All of these are, in principle, candidates for a genetic dissection via QTL mapping. We present here a linkage map of 28 codominant and 10 dominant markers in the Bombina genome. In an F2 cross, markers that were mainly microsatellites, SSCPs or allozymes were mapped to 20 linkage groups. Among the 40 isolated CA microsatellites, we noted a preponderance of compound and frequently interleaved CA-TA repeats as well as a striking polarity at the 5' end of the repeats.
REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ibib abs l17 2-6

L17 ANSWER 2 OF 6 EMBASE
on STN
ACCESSION NUMBER: 2000434896 EMBASE
TITLE: Inheritance of allozyme loci in Bombina: Second linkage group established.
AUTHOR: **Hofman S.**; Szymura J.M.
CORPORATE SOURCE: S. Hofman, Department of Comparative Anatomy, Jagiellonian University, Ingardena 6, 30-060 Krakow, Poland. hof@zuk.iz.uj.edu.pl
SOURCE: Biochemical Genetics, (2000) 38/7-8 (267-274).
Refs: 31
ISSN: 0006-2928 CODEN: BIGEBA
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 021 Developmental Biology and Teratology
022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Segregation and linkage relationship of nine allozyme loci, which are fixed for alternative alleles in the European fire-bellied toads, Bombina bombina and B. variegata, were studied using artificial F1 hybrids to obtain backcross and F2 progeny. Alleles coding for electromorphs at nine loci (Ldh-1, Mdh-1, Idh-1, Ck, Ak, Gpi, Aat-1, Np, and G6pd) showed Mendelian ratios. Two of the loci, Ak and G6pd, were found to be closely linked (2 cM apart); the other loci assorted independently.

L17 ANSWER 3 OF 6 BIOSIS
ACCESSION NUMBER: 1996:402232 BIOSIS
DOCUMENT NUMBER: PREV199699124588
TITLE: Progress evaluation of HIV/AIDS psychological consulting.
AUTHOR(S): Perkins, Helen [Reprint author]; Laski, M.; **Hofman, S.**
CORPORATE SOURCE: Rosales 2571 - 5 D, 1636 Olivos P.B.A., Argentina

SOURCE: ELEVENTH INTERNATIONAL CONFERENCE ON AIDS. (1996) pp. 503. Eleventh International Conference on AIDS, Vol. Two. One world: One hope.
Publisher: Eleventh International Conference on AIDS, Vancouver, British Columbia, Canada.
Meeting Info.: Eleventh International Conference on AIDS, Vol. Two. One world: One hope. Vancouver, British Columbia, Canada. July 7-12, 1996.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Sep 1996
Last Updated on STN: 3 Sep 1996

L17 ANSWER 4 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:402152 BIOSIS
DOCUMENT NUMBER: PREV199699124508
TITLE: Women, sexuality and HIV/AIDS prevention.
AUTHOR(S): Laski, Marina [Reprint author]; **Hofman, S.**; Perkins, H.; Palma, Z.
CORPORATE SOURCE: Defensa 1865, Haedo, 1706 Buenos Aires, Argentina
SOURCE: ELEVENTH INTERNATIONAL CONFERENCE ON AIDS. (1996) pp. 490. Eleventh International Conference on AIDS, Vol. Two. One world: One hope.
Publisher: Eleventh International Conference on AIDS, Vancouver, British Columbia, Canada.
Meeting Info.: Eleventh International Conference on AIDS, Vol. Two. One world: One hope. Vancouver, British Columbia, Canada. July 7-12, 1996.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Sep 1996
Last Updated on STN: 3 Sep 1996

L17 ANSWER 5 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1994:476618 BIOSIS
DOCUMENT NUMBER: PREV199497489618
TITLE: Teenagers, sexuality and HIV prevention in high schools.
AUTHOR(S): Laski, M. [Reprint author]; Perkins, H.; **Hofman, S.**
CORPORATE SOURCE: Defensa 1865, Haedo, Argentina
SOURCE: TENTH INTERNATIONAL CONFERENCE ON AIDS, INTERNATIONAL CONFERENCE ON STD. (1994) pp. 2) 351. Tenth International Conference on AIDS and the International Conference on STD, Vol. 2; The global challenge of AIDS: Together for the future.
Publisher: Tenth International Conference on AIDS, Yokohama, Japan.
Meeting Info.: Meeting. Yokohama, Japan. August 7-12, 1994.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 31 Oct 1994
Last Updated on STN: 31 Oct 1994

L17 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1994:467417 BIOSIS
DOCUMENT NUMBER: PREV199497480417
TITLE: HIV/AIDS psychological consulting: Prevention and care.
AUTHOR(S): Perkins, H. [Reprint author]; Laski, M.; **Hofman, S.**
CORPORATE SOURCE: Rosales 2571, 1636 Olivos, Argentina
SOURCE: TENTH INTERNATIONAL CONFERENCE ON AIDS, INTERNATIONAL CONFERENCE ON STD. (1994) pp. 1) 409. Tenth International

Conference on AIDS and the International Conference on STD, Vol. 1; The global challenge of AIDS: Together for the future.
Publisher: Tenth International Conference on AIDS, Yokohama, Japan.
Meeting Info.: Meeting. Yokohama, Japan. August 7-12, 1994.

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)

LANGUAGE: English

ENTRY DATE: Entered STN: 31 Oct 1994
Last Updated on STN: 31 Oct 1994

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FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
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L1            88 S HOFMAN S?/AU
L2        205543 S DOUBLE STRANDED DNA OR DSDNA
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L4             2 S L3 AND CAPTUR?
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L6           735 S DNA AND (FORWARD PRIMER AND REVERSE PRIMER)
L7           394 S NUCLEIC ACID AND (FORWARD PRIMER AND REVERSE PRIMER)
L8            21 S (L6 OR L7) AND CAPTUR###
L9            11 S L8 AND (MALEAMIDE OR AVIDIN OR STREPTAVIDIN OR CELLULOSE)
L10            5 S L9 AND (IODINE-151 OR RADIOISTOPE OR FLUOROPHOR## OR FLUORES
L11            9 DUP REM L9 (2 DUPLICATES REMOVED)
L12            9 DUP REM L11 (0 DUPLICATES REMOVED)
L13            5 DUP REM L10 (0 DUPLICATES REMOVED)
L14            0 S L1 AND L6
L15            0 S L1 AND L2
L16            6 S L1 AND DNA
L17            6 DUP REM L16 (0 DUPLICATES REMOVED)
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